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Abstract
Adaptation to environmental heterogeneity is a fundamental aspect in evolutionary biology. A constantly changing environment puts continuous stress on organisms, and causes spatially and temporally varying selection regimes where survival depends on responsiveness. Phenotypic plasticity is an important mechanism enabling this responsiveness, which manifests upon exposure to an environmental stressor and facilitates a more resistant phenotype. Environmental heterogeneity exposure at the adult life stage of an organism produces a plastic response that is important for local adaptation and persistence. Hence, adaptive plasticity is an important mechanism of adaptation to localized environmental variation.

To study short term exposure plasticity, we sampled Northern and Southern populations of Drosophila melanogaster, originating from distinct geographic regions and habitats in eastern North America. To elicit a plastic response these populations were exposed to two environmental variables, temperature and photoperiod, for a short-term (five-day) treatment. Flies that had been exposed to this treatment were then tested for phenotypic stress response using chill coma tolerance, heat shock and starvation resistance assays, all of which act as proxies for fitness. To test their response to the natural environment, the same populations were exposed to outdoor field conditions for a treatment equivalent to that in the lab, after which their stress response to heat and cold tolerance was recorded. Whole genome level plasticity was observed by sequencing the transcriptome of lab flies exposed to the same treatment of crossed temperature and photoperiod regimes as the phenotyped flies; thus, allowing for a complimentary gene expression plasticity study.

Geographic origin and temperature treatment determined the phenotypic stress response for the three stress assays. Photoperiod showed significant interaction with temperature, indicating that D. melanogaster is responding to both cues in order to modify its life-history strategies. The field results showed the Northern population had a faster chill coma recovery time when exposed to extreme cold temperatures relative to the Southern population, where this was not observed, suggesting adaptive cold response plasticity is important in the Northern population's fitness. Lastly, the Northern and Southern populations showed a differential expression plasticity response, which is consistent with the expected patterns based on spatially varying selection.

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SHORT TERM ADULT PLASTICITY IN DROSOPHILA MELANOGASTER AND ITS ROLE IN CLIMATIC ADAPTATION

Vinayak Mathur

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in

Biology

Presented to the Faculties of the University of Pennsylvania

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SHORT TERM ADULT PLASTICITY IN *DROSOPHILA MELANOGASTER* AND ITS ROLE IN CLIMATIC ADAPTATION

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This dissertation thesis is dedicated to my mother, Dr. Geetika Mathur and my father, Dr. Vinod Mathur for inspiring me to be what I am today.

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ABSTRACT

SHORT TERM ADULT PLASTICITY IN DROSOPHILA MELANOGASTER AND ITS ROLE IN CLIMATIC ADAPTATION

Vinayak Mathur

Paul S. Schmidt

Adaptation to environmental heterogeneity is a fundamental aspect in evolutionary biology. A constantly changing environment puts continuous stress on organisms, and causes spatially and temporally varying selection regimes where survival depends on responsiveness. Phenotypic plasticity is an important mechanism enabling this responsiveness, which manifests upon exposure to an environmental stressor and facilitates a more resistant phenotype. Environmental heterogeneity exposure at the adult life stage of an organism produces a plastic response that is important for local adaptation and persistence. Hence, adaptive plasticity is an important mechanism of adaptation to localized environmental variation.

To study short term exposure plasticity, we sampled Northern and Southern populations of Drosophila melanogaster, originating from distinct geographic regions and habitats in eastern North America. To elicit a plastic response these populations were exposed to two environmental variables, temperature and photoperiod, for a short-term (five-day) treatment. Flies that had been exposed to this treatment were then tested for phenotypic

stress response using chill coma tolerance, heat shock and starvation resistance assays, all of which act as proxies for fitness. To test their response to the natural environment, the same populations were exposed to outdoor field conditions for a treatment equivalent to that in the lab, after which their stress response to heat and cold tolerance was recorded. Whole genome level plasticity was observed by sequencing the transcriptome of lab flies exposed to the same treatment of crossed temperature and photoperiod regimes as the phenotyped flies; thus, allowing for a complimentary gene expression plasticity study.

Geographic origin and temperature treatment determined the phenotypic stress response for the three stress assays. Photoperiod showed significant interaction with temperature, indicating that D. melanogaster is responding to both cues in order to modify its life-history strategies. The field results showed the Northern population had a faster chill coma recovery time when exposed to extreme cold temperatures relative to the Southern population, where this was not observed, suggesting adaptive cold response plasticity is important in the Northern population's fitness. Lastly, the Northern and Southern populations showed a differential expression plasticity response, which is consistent with the expected patterns based on spatially varying selection.

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INTRODUCTION

Dynamic changes in the environment lead to variation in the forces of selection over spatial and temporal scales. Organisms exposed to such conditions need to function and survive in these heterogeneous environment. The local population must evolve certain traits that provide it with an advantage in local environmental conditions and that increase its fitness in order to proliferate. Under the influence of natural selection, evolution of these traits is known as local adaptation (Williams 2008). Local adaptation is hindered by gene flow. It follows that the signature of local adaptation patterns in the presence of gene flow indicates the strength of selection due to environmental factors (Kawecki and Ebert 2004). Studying the mechanism of adaptation to the environment provides us with an understanding of the evolutionary history of an organism and with a hint to its future evolutionary trajectory.

One of the processes that plays a role in adaptation to the environment is plasticity. Phenotypic plasticity is the change in the expressed phenotype of a genotype as a function of the environment (Scheiner 1993). This plasticity can be manifested in multiple scenarios: the developmental process, the phenotypes related to physiological change or behavior as well as in environment-dependent gene expression, which may not have any visible phenotypic effects at all (DeWitt and Scheiner 2004). The role of phenotypic plasticity in adapting to natural environments has been extensively studied (Stearns and Koella 1986; Stenseth and Mysterud 2002; Walther et al. 2002), but there are not many clear cases of adaptive plasticity (Morey and Reznick 2000; Trussell 2000). Identifying the plasticity of traits and studying them in multiple environments to show a fitness advantage is necessary in order to document phenomenon of adaptive plasticity, which is vital to

understanding the life history of an organism. Adaptive plasticity is likely to facilitate adaptive evolution on ecological time scales in new environments (Ghalambor et al. 2007).

Conceptual Background

The basis of adaptive plasticity lies in the fact that different environments lead to environmentally sensitive phenotypes produced by given genotypes, and these phenotypes have a fitness advantage in their "home" environment. To investigate the incidence of plasticity, we must understand the concept of Genotype x Environment (G x E) interactions as G x E statistics are a necessary for elucidating the role of plasticity in a population's survival trajectory. The G x E interactions are at the foundation of understanding the function of genes and evolution as a whole and entail mapping a phenotype to a genotype. If there was a one-to-one relationship between the genes and phenotypes that they regulate, our understanding of biological processes would be greatly simplified. In reality, however, there are several genes that may be acting in tandem on a particular phenotype or acting antagonistacally. When exploring genetic effects on phenotype, keeping the environmental effects constant is a requirement in order to reduce confounding effects that may influence the phenotype. Thus, the simplest relation between the variance (V) in phenotypes (p), genetic effects (g) and environmental effects (e) can be explained by the following equation:

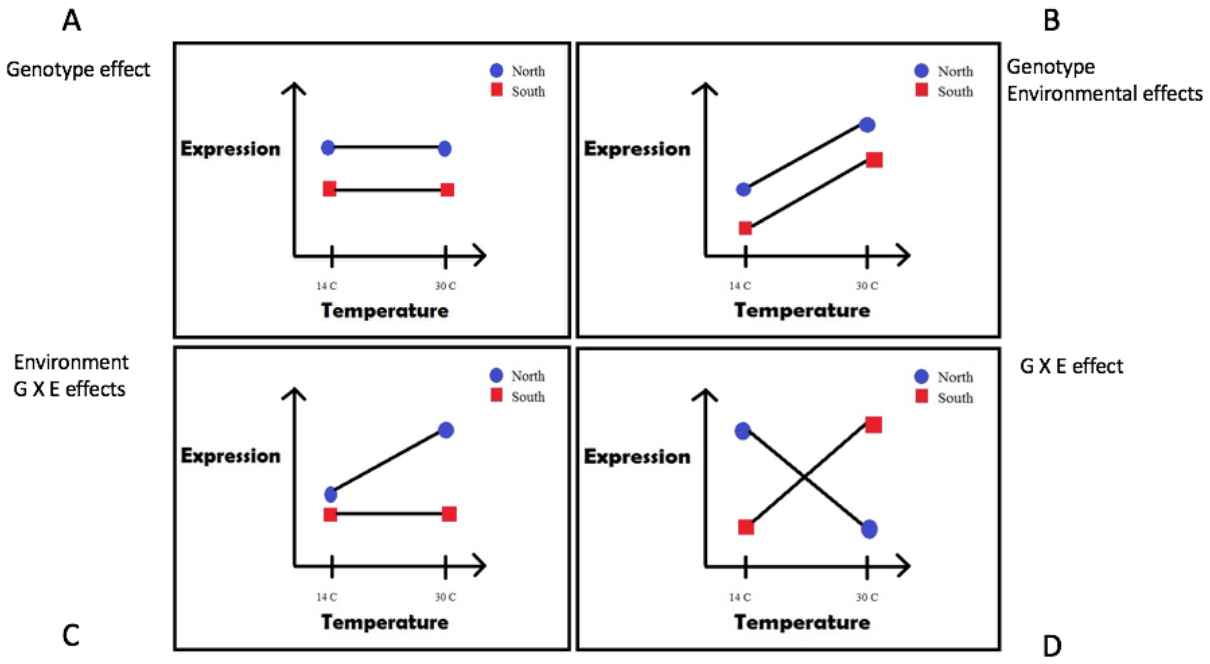
$$V_p = V_g + V_e$$

To further complicate this relationship, the manifestation of different genotypes into one set of phenotypes within one environment is often completely unpredictable from

their manifestation in another environment. This environmental component interacts with genotype providing an independent term (g x e) which influences the phenotypic variation and, most importantly, forms the basis of phenotypic plasticity. The variance term (Scheiner and Goodnight 1984; Via and Lande 1985) can therefore be expanded into:

$$V_p = V_g + V_e + V_{gxe} + V_{error}$$

Using this equation, the reaction norm of the genotype (y-axis), specifically a trait or phenotype (in this case gene expression values), can be plotted against a set of environmental conditions (x-axis). The possible reaction norms are described in the following figure:



Case A - Genetic effects: Flat reaction norms (slope is zero) with significant genetic differences between the populations in the two environments. Reaction norms with slopes

of zero are considered an example of no developmental response to the environment. However, this case is important as it may represent environmental canalization (Wagner et al. 1997). Canalization would result in an adaptive compensatory mechanism that resists the environmental variation. Thus, flat reaction norms may sometimes conceal interesting forms of adaptive developmental plasticity that can underlie trait production (DeWitt and Scheiner 2004).

Case B - Genetic and Environmental effects: Sloped-parallel reaction norms. In this case, genetic differences between the populations and the environmental responses are similar and result in a positive slope. When focusing on expression plasticity, most genes are expected to give this type of pattern of reaction norms indicating the effect of environmentally mediated gene-expression.

Case C - Environment effects and Gene x Environment effects: There is phenotypic variation caused by the environment, but the mean phenotype is same for both populations; hence there is no genetic effect. The differently sloped reaction norms indicate that there is an interaction between genotype and environment. Several modifications of this case are possible when plotting the data from the experiment but the underlying implications remain the same: directional selection caused by the environmental heterogeneity leads to the difference in the slopes of the reaction norms.

Case D – Gene x Environment effects: Two populations show completely opposite phenotypes in two distinct environments. Directional selection causes the variation in phenotype and creates an interesting pattern of potentially adaptive responses. Thus, the

crossed Genotype x Environment patterns leads to a change in the qualitative relationship between trait and fitness (DeWitt and Scheiner 2004).

Model system

Drosophilid species are a model to test for climatic adaptations due to their large range of habitats; from the tropics into the temperate zone in the wild as well as many human dwellings and industries. *Drosophila melanogaster* is a commensal species that has colonized the Americas from tropical Africa and Eurasia a few centuries ago (David and Capy 1988). The range expansion of *D. melanogaster* from the tropics to the temperate environment indicates that these temperate populations are potentially under directional selection of climatic variables. There are several latitudinal clines identified for phenotypes in *D. melanogaster* on multiple continents, e.g. developmental time (James and Partridge 1995), body size (James et al. 1997), fecundity (Mitrovski and Hoffmann 2001), stress resistance (Hoffmann et al. 2002), lifespan and diapause (Schmidt et al. 2005; Schmidt and Paaby 2008), pigmentation (Pool and Aquadro 2007), and desiccation tolerance (Rajpurohit et al. 2013). In addition to phenotypic clines in *D. melanogaster*, latitudinal clines have also been observed for a number of candidate genes and molecular polymorphisms (Schmidt et al. 2000, Gockel et al. 2001, Frydenberg et al. 2003). There is evidence of structured populations of *D. melanogaster* between continents (Schlotterer et al. 2006). Inferred patterns of gene flow have shown that there is no potential for local adaptation and that there is no population structure in *D. melanogaster* within continents (Hale and Singh 1991, Kennington et al. 2003). Latitudinal clines may also be generated

by demography and secondary contact (Bergland et al. 2014, Kao et al. 2015). Nonetheless, functional characterizations of some polymorphisms suggest that at least a subset of the observed clines result from spatially variable selection and adaptation to the heterogeneous climatic environment (e.g., Paaby et al. 2014).

To study the evolutionary response to environmental heterogeneity in an organism it is necessary to work with natural populations that have standing variation, short generation times and relatively quick responses to environmental variables. *D. melanogaster* fulfills these requirements; in addition to having an extensive network of natural populations spread over a latitudinal gradient exhibiting climatic variation.

Phenotypic plasticity in Drosophila

Plasticity in life history traits has been extensively studied and there is research on key traits affecting fitness like adult size, rate of development and rate of juvenile growth (Nylin and Gotthard 1998). In *D. melanogaster*, there is evidence of multiple latitudinal clines for phenotypic traits on several continents that show the same patterns (Partridge et al. 1994; James et al. 1997; Gockel et al. 2001) and thereby indicate the effect of environmental selection on these populations. *D. melanogaster* has been extensively used in studies of stress resistance and the response associated with clinal variation (Gibert and Huey 2001; Ayrinhac et al. 2004). Studies have focused on multiple facets of stress response, including heat tolerance (Hoffmann et al. 2002), cold tolerance (Hoffmann et al. 2005), and desiccation tolerance (Rajpurohit et al. 2013) as well as starvation resistance (Robinson 2000). Most studies identify incidences of developmental plasticity where

larvae are exposed to different environmental stress treatments and their responses are noted as adults. The overall goal of this research is to investigate the effect of short term exposure on adult *D. melanogaster* and observing the response to stress assays based on this acclimation treatment. This short term exposure would result in physiological plasticity that affects performance/fitness. Looking at the effects of short term exposure in distinct populations helps us identify if these populations have different physiological responses. This allows us to test whether the variation in plasticity is consistent with predictions based on local adaptation, e.g. if northern populations are more cold tolerant, then they will exhibit a more robust plastic response to cold exposure.

Expression plasticity

Expression variation as a phenotype: Recent developments in technologies of measuring gene-expression levels have made it possible to conceive gene-expression changes as a phenotypic trait (Nachtomy et al. 2007). RNA-Seq is a recently developed approach to transcriptome profiling that is extremely precise and provides more accurate results than microarray techniques (Wang et al. 2009). With the advent of RNA-Seq, we are able to delve deeper into unresolved questions about the effects gene expression on phenotypes and fitness consequences (Wray 2007).

The selection pressure due to the environment can affect the structural mutations in coding regions of genes or mutations in cis-regulatory regions of genes, which affect the transcription profiles. Wray (2007) hypothesized that cis-regulatory mutations are more likely to affect phenotypic traits and that selection will act more efficiently on these

mutations because transcription is a more dynamic process, i.e. can be more 'fine-tuned', to specific conditions than structural variation. Based on this hypothesis, modulation of transcription of genes under selection can give a faster and more robust response to variables such as temperature change in comparison to the relatively slower selection of favorable alleles in the coding regions, which must then rise to high frequency in the population.

Studies by Levine et al. (2011) and Zhao et al. (2015) have shown evidence of expression plasticity in natural *D. melanogaster* populations in response to developmental temperature. Their results showed significant geographic-origin dependent expression plasticity in individual genes. These studies provide important evidence that there is measurable expression plasticity at the population level that varies in a population-specific way. Using expression variation patterns based on G x E is the key step in identifying candidate genes that are different between populations due to spatially varying selection.

Temperature and Photoperiod as environmental variables

Temperature and photoperiod are certain climatic variables that vary in a predictable manner, closer to the poles temperatures are colder and the variation in day length is larger. For ectotherm species, such as insects, temperature has long been recognized as a major environmental factor responsible for their geographic distribution and species abundance (Ayrinhac et al. 2004). In temperate climates, species have developed a diversity of adaptive mechanisms to tolerate cold conditions during winter, including the occurrence of diapause (Leather et al. 1995), the production of antifreeze

compounds (Graham et al. 2000) and seasonality by development of a critical photoperiod for diapause induction (Tanaka 1992). Photoperiod is an information based cue. Plastic responses may not be caused by photoperiod directly acting as a selecting agent. Instead, photoperiod may act as a sentinel of future changes associated with seasonal light and dark cycles (DeWitt and Scheiner 2004). Response to temperature has been studied extensively, but the influence of photoperiod, which co-varies with temperature, in the environment has not been investigated thoroughly. The interplay of temperature and photoperiod cues is vital for several biological processes in nature. Transcriptome expression responses in populations reared in different temperatures have already been shown (Levine et al. 2011), but the effects of photoperiod on expression have not been documented at the whole genome level.

Short term plasticity response

A differential response in performance and fitness in populations of different geographic origin suggests that selection has modified the plastic response. There are two aspects of this plastic response: developmental plasticity and short term response plasticity. There is suggestion that the developmental plasticity and short term acclimation response are correlated but may have evolved separately from one another (Gerken et al 2015). Furthermore, because environmental parameters can greatly change during the course of an individual's lifespan—on the order of daily fluctuations in temperature through months (Behrman et al. 2015), short term plasticity may be essential to maintaining performance in natural habitats. At the genomic level, exposure to stressful conditions in the adult life stage will require a rapid response that could be modulated by cis-regulatory control. Studies looking at thermotolerance in Drosophila have focused on developmental plasticity

(e.g. Trotta et al. 2006, Fallis et al. 2014), but there is a gap in the understanding of the plastic response based on short term exposure. There are no studies looking at the effect of photoperiod at the genomic level in adult *Drosophila*, even though we know that photoperiod and temperature co-vary in nature and insects are responding to both those cues to modify their life history strategies (Lanciani et al. 1992; Pegoraro et al. 2014). Hence, to study the role of adaptation to environmental heterogeneity we are interested in looking at short term response plasticity at both the phenotypic and genomic level.

Objectives of this study

The goal of this dissertation is to better understand the role of short term exposure plasticity to local adaptation using two geographically distinct populations of *D. melanogaster*. To achieve this goal, I modified the environment of these populations using temperature and photoperiod variables, with the aim of eliciting a physiological plasticity response that affects the performance and fitness of the flies under consideration. Using cold tolerance, heat shock and starvation resistance assays, I quantified the magnitude of differential response in the short term plastic response of each population. I was then able to deduce whether the observed variation in plasticity was consistent with predictions based on local adaptation. For example, one may expect that northern populations of *D. melanogaster* in the US are more cold tolerant then their southern counterparts and that therefore they will exhibit a more robust plastic response to cold exposure. These experimental investigations of plasticity were done under both laboratory and field conditions. Though less common, field experiments are essential for a more profound

plasticity within the context of natural ecological variance. Because we were focused on short term acclimation at the adult life stage and not developmental plasticity, we hypothesized that plastic responses would be modulated by relatively fast cis-regulatory mechanisms, which could be found among a slew of genes affecting phenotypic responses. I tested this by exposing the flies to the same treatment conditions as for the physiological response and observed patterns of gene expression plasticity and the differential response in the two populations. Furthermore, I looked at a series of upstream SNPs that showed High Fst between Northern and Southern populations to investigate the role of spatially varying selection in shaping the observed expression patterns.

Dissertation chapters

In Chapter 1, I characterize the plasticity of stress phenotypes in two geographically varying populations of *D. melanogaster*. I exposed virgin females from the two populations to different temperature and photoperiod regimes to elicit and measure their response to certain stress assays that serve as proxies for fitness. I repeated the experiment with a short term exposure treatment under natural field conditions to compare the stress response with the laboratory assay. In Chapter 2, I document differential expression plasticity in the two populations when exposed to the temperature and photoperiod treatments. To do this, I tested the patterns of genotype by environment interactions in population-level gene expression and categorized the functional role of the differentially expressed genes.

CHAPTER ONE: ADAPTIVE PATTERNS OF PHENOTYPIC PLASTICITY IN LABORATORY AND FIELD ENVIRONMENTS IN DROSOPHILA MELNOGASTER

1.1 ABSTRACT

Identifying mechanisms of adaptation to variable environments is essential in developing a comprehensive understanding of evolutionary dynamics in natural populations. Phenotypic plasticity allows an organism to change its phenotype in response to changes in its environment. In heterogeneous environments, adaptive plasticity may play a major role in adaptation. Here, the plasticity of stress response in two populations (Northern and Southern) of *Drosophila melanogaster* originating from distinct geographic regions and ecological habitats is examined. Flies were given a 5 day short term exposure to high and low temperatures and short and long day photoperiod regimes to elicit a plastic response in chill coma recovery, heat shock tolerance and starvation resistance, all of which act as proxies for fitness in the environment. The short term exposure was replicated in common garden experiment in natural field conditions and the same stress tolerant phenotype assays performed as a comparison with the lab results. Our lab results suggest that geographic origin of the populations determines the phenotypic stress response. Temperature exposure is also a major factor in determining which population is stress resistant. Photoperiod shows a significant interaction response with temperature indicating that the populations are responding to both photoperiod and temperature. The comparative study done in the field gave us an interesting pattern where northern region populations exposed to extreme cold temperatures had a faster chill coma recovery time; a response which was absent in the southern region populations. These findings are evidence for differential cold exposure

response plasticity, which could be an adaptive pattern. This result indicates that there is a short term exposure to temperature and photoperiod has a predictable effect on multiple aspects of fitness. Furthermore, there is a distinct pattern of plasticity specific to the geographic origin of the population, which is consistent with patterns of local adaptation.

1.2 INTRODUCTION

Adaptation to environmental heterogeneity is a fundamental aspect in evolutionary biology. A constantly changing environment puts continuous stress on organisms, and causes spatially and temporally varying selection regimes. For organisms that rely on environmental cues for their development and life-history strategies, anticipation of future environmental conditions can be essential to fitness (eg. Saunders et al. 1989, Stinchcombe et al. 2005). Alternatively, the phenotype expressed by a given genotype can be directly modulated by the specific environmental conditions experienced. Phenotypic plasticity is one such adaptive mechanism, that manifests upon exposure to the environmental stressor and increases stress resistance after exposure (Hoffman and Parsons 1991). It is the change in the expressed phenotype of a genotype as a function of the environment (Scheiner 1993). Evolutionarily, heterogeneous environments are predicted to favor individuals that have an ability to modify their phenotype in response to it (Via and Lande 1985; Scheiner 1993). Thus, adaptive plasticity plays a major role in adaptation to new environments (Ghalambor et al. 2007; Charmantier et al. 2009; Gomez-Mestre and Jovani 2013).

Plasticity can operate at different life-stages of an organism. Developmental plasticity is commonly associated with non-reversible phenotypic changes in response to the developmental environment. In contrast, short term adult plasticity is commonly manifested as a reversible response to short term exposure to specific changes in the environment (Wilson and Franklin 2002; Fischer et al. 2003); this short term response represents a form of acclimation, a physiological response resulting from sensory detection of environmental change and subsequent gene-regulated change in phenotypic expression

(Wilson and Franklin 2002). Basal thermotolerance and acclimation to environmental heterogeneity are both important for local adaptation (Gerken et al. 2015). Thus, the adaptation to novel or fluctuating environmental conditions may reflect a combination of genetically determined basal tolerance levels and the ability to respond via plasticity over short time scales (Nyamukondiwa et al. 2011).

Climate varies predictably over various spatial and temporal scales, and this environmental heterogeneity can result in distinct selection regimes that result in an adaptive response. There is substantial evidence that climate plays a major role as a selective agent on phenotypes of natural populations through adaptation (Grant and Grant 1995, 2002; Bradshaw and Holzapfel 2001). In addition to this, phenotypic plasticity is considered to be one of the major forces involved in adaptive phenotypic changes in response to climatic selection (Przybylo et al. 2000; Réale et al. 2003; Charmantier et al. 2009). Yet amongst the various climate change models that predict species responses, few include adaptive evolution and phenotypic plasticity as deterministic factors (Vedder et al. 2013). Hence, models that do include evolutionary genetics and plasticity are critical in order to improve our predictions of species distributions and population dynamics in a changing environment (Chevin and Lande 2010).

Drosophila melanogaster is an excellent model to examine the dynamics of climatic adaptation as it is a genetic model organism and has a large natural habitat range, which extends from the tropics to the temperate zone. *Drosophila melanogaster* is a commensal species that has colonized the Americas from tropical Africa and Eurasia a few

centuries ago (David and Capy 1988). There are several latitudinal clines identified for phenotypes in *D. melanogaster* on multiple continents, e.g. developmental time (James and Partridge 1995), body size (James et al. 1997), fecundity (Mitrovski and Hoffmann 2001), stress resistance (Hoffmann et al. 2002), lifespan and diapause (Schmidt et al. 2005; Schmidt and Paaby 2008), pigmentation (Pool and Aquadro 2007), and desiccation tolerance (Rajpurohit et al. 2013). In addition to phenotypic clines in *D. melanogaster*, latitudinal clines have also been observed for a number of candidate genes and molecular polymorphisms (Schmidt et al. 2000, Gockel et al. 2001, Frydenberg et al. 2003). Similarly, population level sequencing has demonstrated the pervasive nature of latitudinal clines across the genome (e.g., Kolackowski et al. 2010, Fabian et al. 2012, Bergland et al. 2014) Genomic differentiation has even been seen at the geographical microscale level in the 'Evolution Canyon', Israel based on the different microclimate on opposing canyon slopes (Hubner et al. 2013) The presumed absence of population structure in D. melanogaster suggests that these clines are driven by natural selection and local adaptation; however, latitudinal clines may also be generated by demography and secondary contact (Bergland et al. 2014, Kao et al. 2015). Nonetheless, functional characterizations of some polymorphisms suggest that at least a subset of the observed clines result from spatially variable selection and adaptation to the heterogeneous climatic environment (e.g., Paaby et al. 2014).

While mechanisms generating the observed clines are largely unknown, temperature is a primary determinant of performance and fitness; many clines for thermal related traits have been identified (e.g. Hoffmann and Watson 1993; Guerra et al. 1997; Hoffmann et al. 2002). Temperature plasticity has also been extensively studied in *D.*

melanogaster (Ayrinhac et al. 2004; Hoffmann et al. 2005b) Photoperiod has been shown to have a role to play in stress tolerance and life-history traits in Drosophilid species (Hori and Kimura 1998; Vesala et al. 2012; Bauerfeind et al. 2014), yet many studies looking at the effect of temperature ignore photoperiod as key co-varying variable. In the natural world, temperature and photoperiod co-vary and are of importance in affecting life history traits. (Lanciani et al. 1992; Bradshaw and Holpzafel 2001, Pegoraro et al. 2014).

Long-term acclimation to certain environmental variables, like temperature and photoperiod, provides these organisms with the stress tolerance necessary to deal with potential environmental extremes especially in the face of global climate change. Furthermore, because environmental parameters can greatly change during the course of an individual's lifespan—on the order of daily fluctuations in temperature through months (Behrman et al. 2015), short term plasticity may also be essential to maintaining performance in natural habitats. Ectotherms can use the reversible, short term acclimation response by adjusting their behavior or physiology in response these short term fluctuations (Gerken et al. 2015).

The role of adaptive phenotypic plasticity must also be placed in a realistic ecological context as laboratory assays may not capture the full range of relevant environmental variance (Kristensen et al. 2007; Vanin et al. 2012) Thus, a combination of lab and field based assays can generate a more comprehensive picture of the role of plasticity in the adaptive response to the environmental heterogeneity.

Based on our understanding of short term plasticity and its role in local adaptation to climate we can make the following predictions: Short term exposure to environmental heterogeneity results in physiological plasticity that affects performance and fitness. Populations that are locally adapted will show a variation in their short term plastic response. This variation in plasticity must be consistent with predictions based on local adaptation, for example, if northern populations of *D.melanogaster* are more cold tolerant, then they will exhibit a more robust plastic response to cold exposure.

To explore the potential role of short term, adult plasticity in the adaptation to distinct climatic environments, we manipulated the temperature and photoperiod and exposed *melanogaster* adult females from two distinct climatic environments to these treatment conditions for a short term duration of five days. We utilize a combination of laboratory and field based assays to comprehensively examine the difference in plastic responses to environmental conditions. Our results demonstrate that (1) short term exposure of adults to various temperatures and photoperiods has a pronounced and predictable effect on multiple aspects of fitness, (2) geographically disparate populations exhibit distinct patterns of plasticity and (3) these patterns of differential plasticity are consistent with local adaptation to thermal regime. Together, our results suggest that the short term, physiological acclimation to temperature and photoperiod in this species is modulated by natural selection and may represent an important component in the suite of traits underlying adaptation to environmental heterogeneity in this species.

1.3 METHODS

Collection and maintenance

Natural populations of *Drosophila melanogaster* were collected from both northern and southern regions of the east coast of the US by direct aspiration and established as isofemale lines. Northern populations were collected in October 2009 from Bowdoin, Maine (44.05 ºN, 69.97 ºW) and Shoreham, Vermont (43.89 ºN, 73.31 ºW); southern populations were collected in July 2010 from Jacksonville, Florida (30.33 ºN, 81.65 ºW) and Homestead, Florida (25.46 ºN, 80.47 ºW). The populations were released into insect rearing cages (30 X 30 X 30 centimeters) provided with standard cornmeal food. The cages were established with 40 isofemale lines from each population per region (N = 80 isofemale lines for the northern region, 80 isofemale lines for the southern region). The flies were allowed to interbreed for five generations under standard laboratory conditions (25˚C, 12L:12D). Subsequently, four replicate cages per region were constructed and maintained as independent cultures, thus serving as experimental replicates.

To construct biological replicates, additional populations from the northern and southern regions were collected as before. Isofemale lines were collected in October 2010 from Harvard, Massachusetts (42.5 ºN, 71.58 ºW) and Bowdoin, Maine (44.05 ºN, 69.97 ºW) for the northern region and in July 2010 from Macon, Georgia (32.84 ºN, 83.63 ºW) and Homestead, Florida (25.46 ºN, 80.47 ºW) for the southern region. As was done previously, population cages were created using 50 isofemale lines per population per region (N=100 lines for the north, 100 lines for the south) and allowed to interbreed under standard laboratory conditions for 5 generations. Subsequently, two replicate cages

per region were established and maintained as independent cultures thereafter. Thus, in total there were six experimental population cages per region, comprised of two independent, biological replicates as well as experimental replicates.

Laboratory manipulations

The experimental conditions in the laboratory environment consisted of four treatment combinations in which the temperature and photoperiod were manipulated using an orthogonal design. Flies were exposed to two temperatures: 29°C (hot treatment) and 14°C (cold treatment). An upper limit of 29°C was chosen as it is the highest temperature that does not cause temporary male sterility in laboratory culture (Chakir et al. 2002; Vollmer et al. 2003). The lower temperature was set at 14°C for the cold treatment to avoid expression of reproductive dormancy, which is elicited when flies are exposed to temperatures less than 13°C (Emerson et al. 2009). In addition, flies were exposed to one of two photoperiod regimes, long day (LD, 15L: 9D) or short day (SD, 9L: 15D), representing the extreme seasonal photoperiods for the study site (Philadelphia, PA, USA).

To generate experimental flies, embryos were collected over a 24h period from each of the twelve experimental cages and cultured at 25°C and 12L: 12D. Upon eclosion, virgin females were collected, sorted into groups of five, and held under control conditions for 24h before randomly assigning to one of the four experimental treatment combinations: 29°C LD, 29°C SD, 14°C LD, 14°C SD. Flies were then exposed to the appropriate temperature and photoperiod for a period of 5d, and subsequently transferred into glass vials to perform stress tolerance assays.

Field experiment

In addition to evaluating the effects of short-term exposure to temperature and photoperiod in the laboratory, we examined the effects of exposure to variable field conditions on the stress response. Field experiments were conducted over a period of three years from 2012 – 2014 during the agricultural growing season from April to October. Flies were cultured as before: embryos were collected over a 24h period from each of the twelve experimental cages and cultured at 25°C and 12L: 12D under control conditions. Upon eclosion, virgin females were collected and held at 25°C for 24 hour period before releasing them into outdoor cages consisting of 30 centimeter cube polyester mesh. The floor of each cage was layered with grass and leaf litter and provided with 25 ml of water in a beaker and 50 ml of solidified cornmeal food. Each cage was randomly placed in an experimental garden on the University of Pennsylvania campus in areas without direct exposure to solar radiation. The outdoor treatment lasted for a period of five days, consistent with the treatment duration in the lab experiments. The temperature was recorded using an iButton® device (Maxim Integrated) and the day length data for the five-day treatment was recorded from the National Weather Service website (http://www.weather.gov/) for Philadelphia, PA. At the end of the five-day treatment, each fly was collected by direct aspiration. The flies were brought back into the lab and we performed two thermal stress assays: heat shock survival and chill coma recovery.

There were 11 experimental data points in all and replication was maintained by placing multiple north and south region populations in the outdoor treatment conditions. If individuals exhibited short-term plasticity and physiological responses to temperature or photoperiod, we hypothesized a positive association between exposure to these

experimental variables and performance in stress assays. Differential responses between temperate (north) and subtropical (south) populations indicate that patterns of short-term, adult physiological plasticity may be shaped by natural selection and contribute to local adaptation to climatic conditions.

Phenotypic assays

To evaluate the plastic response associated with temperature and photoperiod, we conducted three stress tolerance assays that have been widely used in the examination of climatic adaptation in Drosophila: heat shock resistance, chill coma recovery time, and starvation tolerance.

Chill coma assay

Low temperatures can elicit similar thermal stress response in *Drosophila* as heat shock, as the flies go into a reversible chill induced coma (David et al. 1998; Gibert and Huey 2001). Chill coma recovery is ecologically relevant, as faster recovery from the inactive state may lead to higher levels of foraging, mate location and reproduction (Fischer et al. 2010).

To evaluate chill coma recovery time, experimental flies from both the laboratory manipulations and field assays were exposed in groups of 5 in empty glass vials to 0°C for a period of 2h (David et al. 1998). Subsequently, vials were transferred to room temperature and allowed to recover; recovery events, defined as the active return to an upright posture, were observed and analyzed using digital video recording (Total N = 1678).

Heat shock resistance

Sudden increases in temperature in nature make adult *Drosophila* highly susceptible to thermal stress (Daahlgaard and Loeschke 1997). Exposure to these high temperatures leads to a heat shock response, which involves expression of molecular chaperones (Feder and Hoffman 1999) and a hardening response when exposed to temperatures above 37°C, with prolonged exposure affecting future survivability (Krebs and Loeschcke 1994). Thus this assay is a measure of robustness to rapid exposure to high temperatures.

Experimental females from both the laboratory manipulations and field experiments were transferred in groups of 5 to glass vials. These vials were then immersed in a 37°C water bath for a period of 2h (Schmidt and Paaby 2008). After the exposure to high temperature, flies were transferred to new vials containing standard medium and allowed to recover at room temperature for one hour. Subsequently, the survivorship of each vial was recorded by counting the number of flies that were still alive (total N = 2059).

Starvation assay

Starvation resistance is associated with metabolic pools and storage (Chippindale et al. 1996; Djawdan et al. 1998; Harshman et al. 1999) and influences survival during adverse conditions when food is scarce (*e.g.* winters in temperate regions, (Mitrovski and Hoffmann 2001). Exposure to cold temperatures results in changes in cell membrane lipid composition (Hazel 1995; Overgaard et al. 2005) and starvation tolerance and cold tolerance may be correlated (Hallas et al. 2002; Hoffmann et al. 2005a). Photoperiod may also play a role in modulating phospholipid fatty acids (Ohtsu et al. 1998; Hodkova et al. 2002), which are associated with starvation tolerance.

To examine the starvation tolerance response, virgin female flies in groups of 5 were transferred to glass vials containing a water soaked (1 mL) cotton ball (Huey et al. 2004). The vials were then placed under control conditions of 25°C, 12L: 12D and mortality was recorded every 3h until all flies had died. (Total N = 1658). The starvation tolerance assay was only performed on experimental flies from the laboratory manipulations.

Statistical analysis

Analyses were performed using JMP v.10 (SAS Raleigh, NC, USA). To examine the among population variation in chill coma recovery and starvation tolerance, a three-way nested ANOVA was performed, with population, temperature and photoperiod as fixed factors. For the heat shock assay we performed a nominal logistical regression, modeling the effects of population, temperature and photoperiod on the log odds of survivorship.

Temperature data were recorded every 10 minutes for all field assays; these data were subsequently used to calculate mean temperature, absolute maximum temperature, absolute minimum temperature, temperature range, mean maximum temperature, mean minimum temperature and the mean temperature difference across the five-day field exposure for each assay. Day length data was obtained from the National weather service (http://www.weather.gov/) for Philadelphia, PA. A Principal Components Analysis (PCA) was performed to determine which environmental variables exhibited the strongest association with variation observed in the stress tolerance assays.

PC1 explained 69.7% of the variation, which directed further analysis. To test which of the recorded experimental variables were correlated with PC1, we performed a

multiple regression between the variables observed over the 5 day treatment period (Zitko 1994): Tavg (average temperature), Tmax (maximum temperature), Tmin (minimum temperature), Tvar (range of temperature), Tmax_avg (average maximum temperature), Tmin_avg (average minimum temperature) and Delta_T (difference between Tmax_avg and Tmin_avg). The regression was also run independently for each geographic region to determine if the same environmental variables exhibited parallel associations. An ANOVA, with population and minimum temperature as the main effects, was performed for the heat shock and chill coma responses.

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1.4 RESULTS

Laboratory assays

We examined the role of physiological acclimation at the adult life stage of *D.melanogaster* on variation in the stress response between Northern and Southern populations of *D. melanogaster* exposed to distinct temperature and photoperiod regimes. We had predicted that if the perception of environmental conditions and subsequent physiological modification represents a component in the adaptive response to local environmental conditions then we will observe the Northern region populations to be more stress tolerant.

Chill coma recovery

As expected, temperature and geographic region were significant predictors of patterns of chill coma recovery in both populations (Table 1.1): flies from Northern region

populations recovered faster than the flies from Southern region populations, and exposure to low temperature also resulted in faster recovery from cold at both long and short day photoperiods (Fig 1.1 A,B). However, the effects of temperature and region exhibit a significant interaction, and also varied significantly with photoperiod (Table 1.1). Populations from the two geographic regions were phenotypically identical when exposed to short day photoperiods at both high and low temperatures, but were significantly distinct when exposed to long day photoperiods (Fig 1.1 A, B; Table A.2). Similarly, photoperiod had no effect on chill coma recovery in the low temperature treatment, but when exposed to high temperatures, exposure to short days resulted in a decrease in recovery time relative to exposure to long days (Table A.2); the combination of high temperatures and long day photoperiods is also the treatment combination that resulted in the largest observed difference between geographic regions. Together, these results demonstrate that both short term exposure of adults to distinct photoperiods and temperatures results in physiological plasticity that has a significant effect on the response to cold. Furthermore, the responses between temperate and subtropical populations are qualitatively distinct in some treatment combinations.

Flies under low temperature conditions showed faster recovery to chill coma. This could be explained by direct influence of genes that respond to the cold acclimatization treatment (Goto 2001; Sinclair et al. 2007). The effects of photoperiod and cold tolerance in *Drosophila* indicate that short photoperiod conditions increase the cold tolerance in the species (Lanciani et al. 1992). We also see that the overall chill coma recovery time is lower under short day conditions than when the flies are exposed to longer day lengths.

There is evidence to suggest that flies developing under short photoperiod conditions exhibit faster recovery times in the adult life stage than those placed under long photoperiod conditions during development (Pegoraro et al. 2014). This study (Pegoraro et al. 2014) focuses on developmental plasticity, which is correlated with cold resistance after short term acclimation response to rapid cold hardening (Gerken et al. 2015). Short day conditions are associated with the onset of winter and the flies maybe responding to the photoperiodic cues along with the lower temperatures, to prepare for long term exposure to the onset of cold conditions.

Heat shock assay

As was observed with response to cold, both temperature exposure and geographic region had a significant effect on survivorship following exposure to high temperature; photoperiod also affected tolerance to heat. However, the main effects also exhibited significant interactions, including a three-way interaction between temperature, photoperiod, and geographic region (Table 1.1). Under exposure to long day photoperiods, flies from the northern and southern regions exhibited parallel response to temperature, with short term exposure to high temperature resulting in elevated heat tolerance, and the northern populations again being more stress tolerant (Figure 1.1 C). A very different pattern was observed, however, under short day photoperiods: tolerance to heat was equivalent between northern and southern regions when exposed to low temperature, but survivorship was significantly distinct between regions when flies were exposed to elevated temperature (Figure 1.1D). The non-parallel responses between regions demonstrated that Region, temperature and photoperiod all had significant effects when

flies were exposed to heat shock. The temperature × region and temperature × photoperiod interactions are both significant along with the three-way interaction term (Table 1.1).

A previous study on developmental plasticity of thermal tolerance has not found differences in population plasticity response in their adult life stage based on rearing temperature (Cooper et al. 2012). In the long day photoperiod conditions in our experiment, there are similar levels of plasticity in heat shock response for both populations as we move from lower to higher temperature treatment. But for the short day treatment we can observe a significant genotype by environment interaction effect where the Southern population shows a drastic plastic response in the opposite direction trend as compared to the long day treatment. This suggests that photoperiod has a role to play in the plasticity response of these flies. Longer photoperiods have been associated with increased heat tolerance phenotype in the fly *Protophormia terraenovea* (Fischer et al. 2012), which is similar to the pattern that we observe in our data. One possible explanation of this pattern could be that progressively longer photoperiods are indicative of oncoming summer, and thus trigger increased heat resistance.

The effect of photoperiod is seen to be masked by temperature when looking at effects of developmental plasticity to heat tolerance (Bauerfeind et al. 2014). In their study, (Bauerfeind et al. 2014) found that cooler developmental temperatures reduced heat tolerance. Along the same logic, we see an increase in heat tolerance in the flies exposed to the heat treatment. Exposure to heat stress in young *D. melanogaster* adults impacts the physiological stress response later in life by increasing heat tolerance in future exposures (Kristensen 2003). Either there is increased ability to react to future heat stress or the heat exposure causes regulational changes of heat shock proteins (Hsp70),

which enables the flies to respond better when exposed to stressful conditions repeatedly. There are difference in results between studies looking at developmental plasticity and short term exposure plasticity for the heat shock response. Additionally, our results indicate that photoperiod, which has previously been overlooked in studies of thermo-tolerance, could have a major role to play in how *D.melanogaster* responds to changes in environmental conditions.

Starvation tolerance assay

As with both chill coma recovery and heat tolerance, starvation tolerance was distinct between geographic regions; it was also affected by both temperature and photoperiod (Table 1.1). Also similar to the patterns observed for the temperature tolerance phenotypes, resistance to starvation demonstrated significant interactions of temperature with region and photoperiod. Following exposure to low temperatures at both long and short day photoperiods, flies from northern and southern regions exhibited qualitatively identical starvation tolerance. However, the short term exposure to high temperatures resulted in a significant difference between regions, with the northern flies exhibiting significantly higher tolerance (Fig 1.1 E,F; Table A.2). This demonstrates that higher temperature exposure negatively effects starvation tolerance in the Southern region populations.

Thus, starvation tolerance showed a significant effect of geographic origin with the Northern population having higher starvation tolerance than the Southern one. There is evidence of trade-off between starvation tolerance and cold treatment (Hoffmann et al. 2005a). Our results illustrate this trade-off in that the flies at low temperature treatment

show higher starvation resistance in both the populations as compared to the higher temperature treatment. Exposure to higher ambient temperatures lowers starvation resistance in *Bicyclus anynana*, a pattern that appears to be consistent for other ectothermic species (Pijpe et al. 2007), including the *D. melanogaster* in our experiment. Overall, temperature is known to affect the metabolic rates and water balance involved in starvation resistance (Prakash et al. 2013). Cold exposure has been shown to affect cell membrane lipid composition (Cossins et al. 2002). This phenomenon has been observed in several species of *Drosophila* from temperate climates (Ohtsu et al. 1998). It is also known that flies from distinct locations differ in their starvation tolerance response based on their propensity to store body lipid (Sisodia and Singh 2010, Ballard et al. 2008).

Higher metabolic rates are associated with reduced starvation resistance and in *D. melanogaster* they have been correlated with exposure to short day photoperiod conditions (Giesel et al. 1990). Though most patterns appear to be driven by temperature, we did observe a reduction in starvation tolerance for the Southern fly population. Exposure to colder temperatures results in higher starvation resistance, irrespective of the origin of the population or the photoperiod regime.

Field experiment

Tavg ($R^2 = 0.95$) and Tmin ($R^2 = 0.94$) were the two variables with the highest observed regression coefficients. No significant difference between regions was observed in the highest R^2 coefficient values for Tavg or Tmin, and thus all populations were analyzed together. We selected Tmin as the predictor variable as it is a more ecologically relevant parameter than the average temperature (Kelty and Lee 2001). Each field

experimental data point has a unique Tmin value as well as other associated environmental variables, namely temperature and photoperiod

In the 11 independent assays conducted in the field, the minimum temperature experienced by flies over the 5day field exposure had a significant effect on the time to recovery from chill coma; as expected, exposure to lower temperatures in the field resulted in a faster time to recovery from subsequent cold exposure in the laboratory based assay (Table 2.1; Fig 2.1). Additionally, while the main effect of geographic region was not significant, a significant interaction between minimum temperature and region was evident. Flies from Northern region populations exhibited a stronger response to temperature exposure, and recovered significantly faster as a function of decreasing temperature in the field. In contrast, flies from the Southern region populations exhibited a shallower response to field temperature and did not recover as quickly when exposed to low temperatures in the field. This again demonstrates a non-parallel response associated with geographic origin, in which patterns of physiological acclimation vary predictably among populations.

In the heat shock assay, there is no effect of environmental exposure in the field on the subsequent survivorship of the flies after treatment. This is true for both populations from the Northern and Southern regions, which also do not have significant main effects or interactions. There is a positive correlation between Tmin and higher survivorship, though it is not significant. The heat shock response was fairly similar for both populations suggesting that there is trait specific plasticity in this system. Similarly, the trend line seems to indicate that an increase in the minimum temperature causes no change in response for the two populations.

. These differential patterns from the two populations in the chill coma recovery assay are indicative of adaptive plasticity in flies from the Northern population, but not the Southern. This plasticity in the cold response is an indication of potential cold adaptation in the Northern populations. Similar to the results seen in the lab, field experiments showed that temperature was a major driver of the response in populations from both North and South regions. In the lab, observed that chill coma recovery reaction lines were parallel in their response and that the lower temperature treatment resulted in faster recovery times for the populations. We see a similar observation in our field results, where faster recovery times correspond to lower Tmin values experienced in the field treatment. For the heat shock assay, higher temperature exposure was significantly correlated with higher survivorship in the lab, but not the field. Though not significant, the reaction lines in the field experiment are similar to those in the lab in that at least they both show parallel responses for populations from Northern and Southern regions.

1.5 DISCUSSION

Our results demonstrate a significant effect of geographic origin of the populations in which flies from the temperate environments exhibit better performance under thermal stress in comparison to populations from subtropical habitats. The short term exposure to different temperature and photoperiod conditions results in a significant response for the observed traits, demonstrating that short term acclimation has a large effect on all measured aspects of stress tolerance.

Both Northern and Southern region populations showed plastic responses to the measured traits but we observed a differential response which was trait specific. Chill coma recovery assay and the heat shock tolerance assay both had parallel reaction norms for the Long day photoperiod treatment at both temperatures. Under short day conditions we see evidence on non-parallel reaction norms for the Southern population and evidence of the Genotype x Environment interaction. Southern region populations perform much worse at heat tolerance when exposed to short day conditions, which these populations do not get exposed to in Southern United States where the day length is more consistent over the year. Similarly for the starvation tolerance assay at high temperatures, the Southern region populations show a sharper decrease in tolerance to starvation stress whereas the Northern population perform better and have a more consistent tolerance response.

The results we observed seem to suggest the populations that are better able to perceive environmental change have evidence for elevated fitness. The Northern region population are exposed to extremities of temperature changes over the season and even on daily time scales. They are also experiencing larger fluctuations in the day length throughout the year as opposed to the more stable environment in sub-tropical latitudes.

The data indicates that the Northern population is more heat tolerant and cold tolerant under certain environmental parameters, which is similar to patterns seen in flies from temperate climates in North America under normal lab conditions (Schmidt and Paaby 2008). Research on thermal clines in *Drosophila melanogaster* populations in eastern Australia show an increased thermal tolerance seen in tropical populations (Hoffmann et al. 2002). The difference in the results for the two continents could be explained by the severity of the winter in North America relative to Australia. Exposure

to higher fluctuations in temperature could potentially be the cause of the different pattern seen. Additionally the colonization of Australian continent is relatively recent – less than 100 years ago (Bock and Parson 1981), which could cause the differences in the heat tolerance cline.

For both the chill coma and heat shock assay, exposure to temperature regimes increased the flies' stress tolerance. The observed patterns of starvation tolerance could be explained in terms of temperature affecting metabolic rate. The effects of photoperiod exposure are more nuanced than those of temperature exposure. Short day treatments decreased the time needed to recover after chill coma exposure in both populations, which could indicate that the flies are responding to the cues similar to those signaling the onset of winter. Higher cold resistance is a logical pattern to expect in such conditions. Similarly for heat shock, exposure to a longer photoperiod may indicate summer's onset, and could potentially signal heat shock resistance. The effect of photoperiod was unclear for starvation resistance and may just be closely related to cold shock tolerance.

Our field experiment results illuminate the responses to conditions in nature and test the ecological relevance of the data. Studies that focus on modulating temperature and other environmental parameters in laboratory conditions aim to interpret the results in the context of the environmental conditions that the organism is expected to meet in the field. However, the laboratory and field results may not always be equivalent (Kristensen et al. (2008), Vanin et al. (2012). Our field results show the similar non – parallel response reactions norms that had been observed for in the lab for the different measured traits. The patterns observed validate that there is a differential response in these

populations of different geographic origin which is trait specific and shows the underlying evidence of local adaptation. Similar to the lab, the Northern region populations are more responsive to the cold exposure treatment and show a faster chill coma recovery time when given an extremely cold temperature exposure in the environment. The similar response is missing in the southern region populations. This pattern makes sense in terms of local adaptation to the temperate climate that the northern populations are exposed to throughout the year. This acclimation response appears to be shaped by selection as an adaptive strategy against cold exposure in temperate environments.

There are several studies that focus on the differences in thermal tolerance between temperate and tropical populations (e.g. Chown and Terblanche 2006; Sgrò et al. 2010). Still, it is unclear based on the literature whether there is a trend in which populations are more plastic. We have managed to show that there are plastic responses in the way *Drosophila melanogaster* from northern and southern region populations respond to temperature and photoperiod variation. Additionally, these plasticity patterns are seen in response to natural environmental conditions, indicating that they are a major component of the life history strategy in this species. We have evidence of adaptive plasticity in the chill coma recovery trait that suggests the temperate population is cold adapted to the colder environmental conditions. This plasticity is trait based and the trends are not the same at different environmental conditions but population specific differences do exist. The mechanism of plasticity for these environmental variables is not clear in this species and work needs to be done looking at the genes involved with

differential plastic response in this species. The existence of adult stage plasticity over short term exposure in *Drosophila* make it an ideal candidate to test for the mechanistic principles behind these responses.

TABLE 1.1 Statistical results for planned comparisons looking at the main effects of Region, Temperature and Photoperiod and their interactions on the stress tolerance phenotypes.

Effect tests			Chill coma			Heat shock			Starvation tolerance	
Source	N	DF	SS	F	P	L-R ChiS	P	DF Den	F	P
Region	1	1	180198	4.4865	**0.0343***	21.698	**<.0001***	141.5	6.9767	**0.0092***
Temperature	1	1	34610790	861.7248	**<.0001***	11.375	**0.0007***	1907	224.8572	**<.0001***
Photoperiod	1	1	513	0.0128	0.91	5.780	**0.0162***	1838	15.9171	**<.0001***
Replicate[Region]	2	2	742261	9.2403	**0.0001***	5.023	0.0811	136.5	1.2943	0.2774
Cage[Region, Replicate]	8	8	2641925	8.2222	**<.0001***	52.528	**<.0001***	136.7	0.5549	0.813
Region x Temperature	1	1	2493455	62.081	**<.0001***	20.629	**<.0001***	1907	23.6726	**<.0001***
Region x Photoperiod	1	1	6092861	151.6975	**<.0001***	0.521	0.4703	1838	1.161	0.2814
Temperature x Photoperiod	1	1	290304	7.2279	**0.0072***	10.318	**0.0013***	1851	54.7555	**<.0001***
Region x Temperature X Photoperiod	1	1	125219	3.1177	0.0776	15.829	**<.0001***	1851	0.0101	0.9197

TABLE 1.2 Statistical results for planned comparisons looking at the main effects of Population, Tmin and their interaction on the stress tolerance phenotypes.

Effect tests			Chill coma			Heat shock		
Source	N	DF	SS	F	P	SS	F	F
Region	1	1	0.6	0	0.9954	0.06989091	1.0935	0.3095
Tmin	1	1	247502.17	13.6334	**0.0002***	0.08142389	1.274	0.2738
Region*Tmin	1	1	283860.17	15.6362	**<.0001***	0.00038219	0.006	0.9392

Figure 1.1: Phenotypic response in the laboratory assays

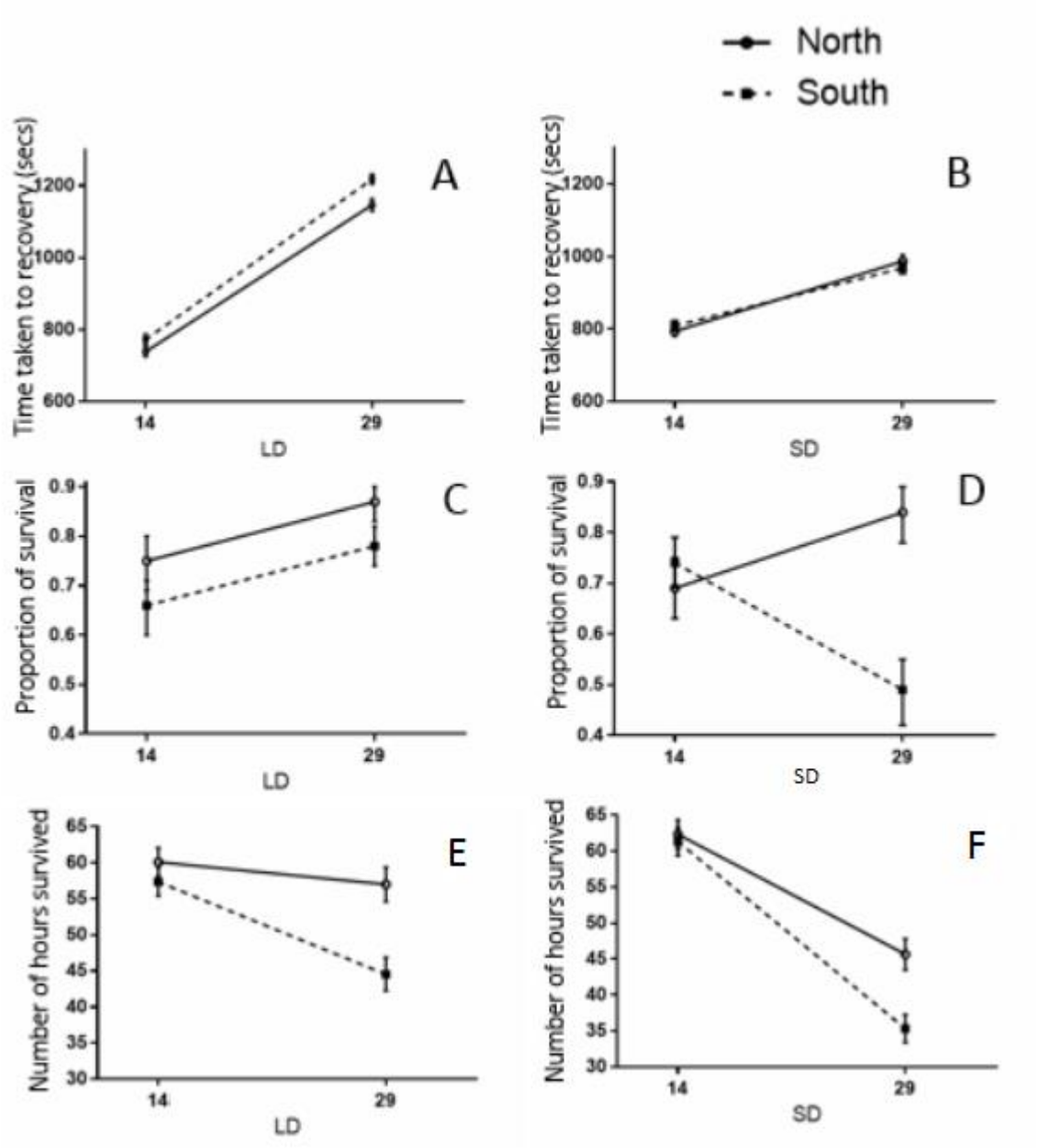
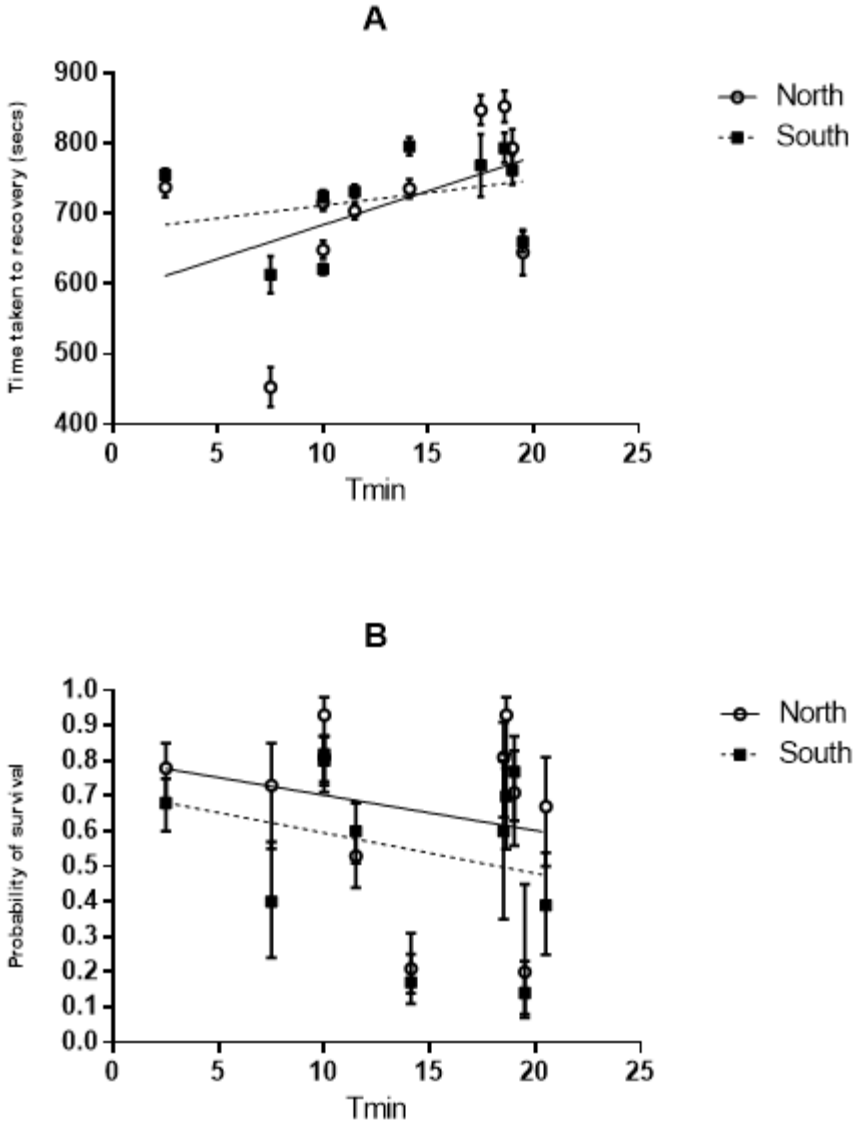


Figure 1.2: Phenotypic response in the field assays



CHAPTER TWO: EXPRESSION PLASTICITY IN RESPONSE TO CLIMATIC VARIABLES IN *DROSOPHILA MELANOGASTER*

2.1 Abstract

Gene expression shows plasticity in response to environmental heterogeneity. These patterns of expression may have been shaped and maintained by natural selection. To test this hypothesis, we investigated two geographically distinct populations of *Drosophila melanogaster* from eastern North America and determined their gene expression patterns in response to certain environmental variables. The populations were given a short-term exposure to unique temperature and photoperiod regimes, after which differential patterns of expression were identified. We observed a differential response of gene expression between the Northern and Southern populations where the Southern population showed increased levels of plasticity in response to both temperature and photoperiod treatments. A differential expression response was also apparent in how the populations responded to specific temperature (Hot and Cold) and photoperiod (Long and Short Day) conditions, providing further evidence for spatially varying selection as the agent driving patterns of gene expression. Functional analysis revealed similar gene ontology categories enriched in both populations. This indicates a parallel mechanism of response at the categorical level and suggests that differences in response plasticity may be attributed to differences in individual genes within a category. We investigated this hypothesis by comparing our dataset with a complimentary dataset detailing the genomic

differentiation in the same Northern and Southern populations. As predicted, we found significant enrichment of SNPs in the upstream region of the differentially expressed genes, indicating that the expression variation is driven by cis-regulatory modulation. This study provides evidence for differential expression response to environmental variables based on the geographic origin of the populations, which is consistent with patterns of local adaptation based on selection due to climatic variables.

2.2 Introduction

Spatially varying selection causes changes in the phenotypic traits and the genotype of a population spread over a large latitudinal range. Plasticity is the change in the expressed phenotype of a genotype as a function of the environment (Scheiner 1993). This phenomenon has been extensively studied at the phenotype level for multiple organisms. (Pigliucci 2001; Whitman and Ananthakrishnan 2009). Plasticity may lead to a change in fitness of an organism and thus could be an important mechanism to escape the effects of short term environmental stress.

Gene expression is one of the traits that shows a wide range of variation in multiple organisms across various environmental conditions (Causton et al 2001, Levine et al. 2011, Richards et al. 2012). Plasticity in gene expression could affect different life history strategies by changing the mechanism to escape stressful conditions. There is evidence of different environments producing a variation in expression that is manifested in certain phenotypic changes (Bochdanovits et al. 2003; Liefting et al. 2009; Sinclair et al. 2007; Swindell et al. 2007). Additionally gene expression plasticity between populations of different geographical origin is evidence of spatially varying selection acting on these populations. (Levine et al. 2011, Zhao et al 2015).

Drosophila melanogaster is a human commensal that colonized the Americas from tropical Africa and Eurasia a few centuries ago (David and Capy 1988). Several phenotypic traits have been identified in *D. melanogaster* that vary clinally in eastern North America (e.g. Coyne & Beecham 1987, Schmidt et al. 2005; Schmidt and Paaby 2008). Population

level sequencing provides evidence of certain clines existing across the genome as well (e.g., Kolackowski et al.2011, Fabian et al.2012, Bergland et al.2014). In some cases these latitudinal clines may have been generated by demography or secondary contact (Kao et al. 2015, Bergland et al. 2015) but there is enough evidence of these observed clines being shaped by spatially varying selection and adaptation to heterogeneous environments. Additionally, there are studies that have observed the effect of environmental stress on gene expression plasticity in wild populations and there is evidence of differential response based on population origins (Telonis-Scott et al. 2013, Levine et al. 2011). High degree of plasticity in gene expression in populations exposed to different developmental temperature indicates that the environment has profound effects on expression norms (Chen et al. 2015). Studying the effects of the environment on expression plasticity at the genome level provides information on the mechanism of response and the functional requirements of these populations.

Exposure to variation in temperature regimes and studying the subsequent phenotypic and genomic changes has been extensively studied in *D. melanogaster*. For ectotherms, temperature plays a key role in controlling developmental rate and morphological traits (Azevedo et al.1996; K. Fischer et al. 2003; Gibert and De Jong, 2001). Photoperiod is another important environmental variable that varies in a predictable manner over spatial and temporal scales. Circadian rhythms are robust in flies and correlate with certain morphological traits (Bauerfeind et al. 2014; Fischer et al. 2012; Lanciani et al. 1990). Changes in photoperiod will have a cascading effect on the gene expression but

currently, studies have only looked at expression at single gene locus in response to modulating photoperiod regimes (Vesala et al. 2012).

To explore the role of the environment on gene expression plasticity *D. melanogaster* adult females from two geographical locations were exposed to two environmental variables, temperature and photoperiod and sequenced to categorize their transcription profiles. We are interested to identify the genes that show patterns of differential expression between the populations indicating their role in adaptation to the heterogeneous environment.

2.3 Methods

Collection and maintenance

Natural populations of *Drosophila melanogaster* were collected from the northern and southern regions of the east coast of the US by direct aspiration and isofemale lines were established. Northern populations were collected in October 2009 from Bowdoin, Maine (44.05 ºN, 69.97 ºW) and Shoreham, Vermont (43.89 ºN, 73.31 ºW); southern populations were collected in July 2010 from Jacksonville, Florida (30.33 ºN, 81.65 ºW) and Homestead, Florida (25.46 ºN, 80.47 ºW). Population cages were set up by pooling the isofemale lines into insect rearing cages (30x30x30 centimeters) provided with standard cornmeal food. The cages were established with 40 isofemale lines from each population per region (N = 80 isofemale lines for the northern region, 80 isofemale lines for the southern region). The flies were allowed to interbreed for five generations under

standard laboratory conditions (25˚C, 12h Light: 12h Dark). Subsequently, four replicate cages per region were constructed and maintained as independent cultures, thus serving as experimental replicates.

Experimental treatment

The experimental lab treatment consisted of four unique treatment conditions varying the temperature and photoperiod with an orthogonal design. The temperatures were 29°C (hot treatment) and 14°C (cold treatment). An upper limit of 29°C was chosen as it is the highest temperature that does not cause temporary male sterility in laboratory culture of this species (Chakir et al. 2002; Vollmer et al. 2003). The lower temperature was set at 14°C for the cold treatment to avoid expression of reproductive dormancy, which is elicited when flies are exposed to temperatures less than 13°C (Emerson et al. 2009). In addition, flies were exposed to one of two photoperiod regimes, Long Day (LD, 15L: 9D) or Short Day (SD, 9L: 15D), representing the extreme seasonal photoperiods for the study site (Philadelphia, PA, USA).

To generate experimental flies, embryos were collected over a 24h period from each of the twelve experimental cages and cultured at 25°C and 12L: 12D. Upon eclosion, virgin females were collected, sorted into groups of five, and held under control conditions for 24h before being randomly assigned to one of the four experimental treatment combinations: 29°C LD, 29°C SD, 14°C LD, 14°C SD. Flies were then exposed to the appropriate temperature and photoperiod for a period of 5 days, and subsequently transferred into glass vials to prepare for sequencing.

RNA preparation and sequencing

We prepared 32 samples for RNA sequencing, which were made up of flies from the 8 population cages placed in 4 unique treatment conditions. 100 females from each of the population and treatment combinations were transferred to glass vials and placed at room temperature for 60 minutes to stabilize expression levels after removing them from the incubator. The flies were transferred to 2ml Eppendorf tubes and immersed in RNAlater solution to stabilize and protect cellular RNA.

Total RNA was extracted using a protocol involving Trizol (Invitrogen) and liquid nitrogen. cDNA library preparation was done using TruSeq RNA Library Preparation Kit v2 (Illumina). Each sample was barcoded for sequencing and run on a total of four lanes using single end reads on the Illumina HiSeq sequencing 2000 platform at the Next-Generation Sequencing Core at the University of Pennsylvania. The raw reads were mapped onto the Drosophila genome using Bowtie2 v2.2.3 software (Langmead and Salzberg 2012). To map the mRNA reads we used TopHat 2.0.12 alignment software (Trapnell et al. 2009). The reads were mapped to reference genome (FlyBase r5.40). We used Cuffdiff 2, a part of the Cufflinks package (Trapnell et al. 2010) to generate differential expression between samples and plotted the data using R-3.1.2 (R Development Core Team 2014) package "cummeRbund" (Goff et al. 2013). There were two samples from the North – Hot and Long Day treatment that did not show a robust pattern of differential expression and we removed them from further analysis (Table B.10). The total number of samples used for the analysis were 30.

Expression patterns

To generate count data we used HTSseq (Anders et al. 2014) to convert every aligned sequence into expression counts for every gene. We calculated the reads with upper quantile normalization and called the expression value (RPKM) from that. Genes were selected that had RPKM > 1 in at least half of the libraries. Volcano plots for genes with differential expression for temperature and photoperiod were generated using native R-3.1.2 software (R Development Core Team 2014) (RPKM>1, FDR< 0.3).

Differential expression analysis

We quantified differential expression using edgeR (version 3.1) (Robinson et al. 2010). We built generalized linear models for each population separately, including temperature and photoperiod conditions and their interaction as predictors of RPKM. For each test, we used an FDR < 0.3 as a cutoff for the differential expression. All chromosome gene locations used in the analysis were downloaded from Flybase. Inversion breakpoints were from Corbett-Detig and Hartl (2012) and the gene lists were tested for enrichment in these inversion regions.

Functional annotation

To perform enrichment analysis on gene sets to find gene ontology terms we used goseq R Bioconductor package (Young et al. 2010). This package takes gene length bias into account (Oshlack and Wakefield, 2009). The subset of enriched gene categories were identified and the *p* values were corrected using the Benjamini and Hochberg procedure (Benjamini & Hochberg 1995), and the false discovery rate threshold was set to be 0.05.

Additionally, DAVID annotation tool (Huang et al. 2009) was used to determine the specific genes in the enriched gene categories.

Determining genomic variation for comparison with expression data

To determine geographic variation in *D.melanogaster* populations from North America we use a database created by Fabian et al. (2012) for comparison with our data from the Northern and Southern populations. They used the same isofemale lines from Maine and Florida as our experiment to determine Fst differentiation between these populations. Based on the genomic data, they had identified SNPs in the 1 Kb upstream region of genes that were highly differentiated using a cutoff of q val< 0.05. After filtering out these genes we were able to compare this list with our data set of differentially expressed genes from the Northern and Southern region populations to create a list of differentially expressed genes with a significantly differentiated upstream SNP. Using a chi-square test, we determined whether each list contained proportionately more genes containing upstream SNPs than non-differentially expressed genes. We also compiled a list of known transcription factor genes from the FlyTF database (Adryan and Teichmann 2006) and compared them to our list of differentially expressed genes with an upstream SNP.

2.4 Results

We performed whole genome transcriptomics for *D. melanogaster* virgin females which had been exposed to different temperature and photoperiod conditions in order to

identify the genes that show differential expression patterns between the high and low latitude populations. We had four experimental replicates for the two populations (North and South) crossed with four experimental treatment combinations (29 ºC LD, 29 ºC SD, 14 ºC LD, 14 ºC SD) to give a total of 32 libraries.

Identifying differentially expressed genes

For the temperature treatment, in the Northern region population there were 434 unique genes that were differentially expressed compared to the Southern region population which had 2518 genes (False Discovery Rate, FDR < 0.03). 2359 genes were differentially expressed in both populations (Table 2.1). When comparing the number of genes upregulated for the hot (29ºC) and the cold treatments (14ºC) for the North population we found identical numbers of genes, 1439 and 1555 differentially upregulated respectively (Table 2.2). For the Southern population there were more genes upregulated in the hot (29ºC) treatment – 2695 genes, as compared to the cold (14ºC) treatment (Table 2.2). For the photoperiod treatment, there were no differentially expressed genes identified in the Northern population, while there were 347 differentially expressed genes identified in the Southern population (Table 2.1). In the Southern population, 139 genes were differentially upregulated in the Long Day treatment compared to 199 genes in the Short Day treatment (Table 2.2). The higher number of differentially expressed genes in the Southern population suggests that these flies may possess higher expression plasticity than their Northern counterparts. Even though the

Northern region flies did not show any differential response for photoperiod, there was a significant photoperiod x temperature response with 14 genes showing differential expression in the Northern population compared to only 2 in the Southern population (Table 2.1).

Testing for functional categories

We performed a GO term enrichment analysis using goseq and DAVID annotation tool to check for functional enrichment of gene categories. For the temperature treatment, the Northern population was enriched in catalytic activity, ion binding, binding, hydrolase activity and nucleotide binding (Table B.3). Catalytic activity (GO: 0003824) is closely associated with transcription factors (Borgonove et al. 2014). For the Southern region population the categories that were enriched were catalytic activity, transferase activity, ion binding, nucleotide binding and nucleoside phosphate binding (Table B.3). Three out of the top 5 GO term categories are shared between the Northern and Southern populations, suggesting they have similar enrichment response to temperature. Using the DAVID annotation tool, we sought out GO categories that would be directly involved in response to temperature in order to better understand the mechanism of the response (Table B.7). In the genes uniquely differentially expressed in the Northern population, structural molecular activity and membrane gene categories were enriched. Cold acclimation leads to production of cyroprotectants which stabilize the membrane structures (Lee, 1991; Lee, 2010). It may also lead to changes in the

phospholipid composition of membranes (Koštál et al., 2003; Overgaard et al., 2008). The role of temperature in influencing the structure of the membranes in *D.melanogaster* is observed in the gene categories enriched for the Northern population. The uniquely differentially expressed genes in the Southern region population was enriched for lipid binding, biological rhythms and immunity response genes (Table B.7). Enrichment for the circadian rhythm and immunity categories was also observed by Fabian et al. 2012 when looking at genomic differentiation in the same Northern and Southern populations. There is evidence that circadian rhythms in insects are closely related to temperature (Pegoraro 2014). The immunity response of genes enriched in the Southern population maybe a function of pathogen diversity and abundance across latitudes (Fabian et al 2012). For the photoperiod category, which was only differentially expressed in the Southern population, there were three GO term categories that showed enrichment – structural constituent of ribosome, binding and protein binding (Table B.3). For the genes that were differentially expressed for the temperature x photoperiod interaction term, 9 out of 14 do not have a known molecular function noted on the Flybase database. However, 6 out of these 9 unknown function genes have protein features related to the immune-induced protein Dim, which are proteins induced during the immune response of *D. melanogaster* (Table B.9). The other 5 genes with a known function are categorized as hydrolase activity (CG14120), cysteine-type endopeptidase activity (CG4847), oxidoreductase activity (Cyp304a1) and insulin receptor binding (Ilp8). Only 2 genes were differentially expressed for the temperature x photoperiod interaction treatment in the Southern population, which are categorized as having zinc ion binding (CG1815) and actin filament binding (Vrp1) functions.

We compared our list of differentially expressed genes within temperature treatments to a previously published study comparing the parallel gene expression in low and high latitude populations of *D. melanogaster* and *Drosophila simulans* (Zhao et al. 2015). Out of the 1546 unique genes that were differentially expressed between high (29ºC) and low (21ºC) developmental temperature treatments in that study (Zhao et al. 2015), we found 537 differentially expressed genes that were shared with our study and that were also in response to temperature (34.7%) (Table B.5). One of the gene categories that was most differentiated in the Zhao et al. (2015) dataset was for transcription factors. The trp gene, within the transcription factor category, was differentially expressed in our Southern population dataset as well. Two other transcription factors, srp and az2 identified by Zhao et al. (2015) were differentially expressed in both of our Northern and Southern populations. Additional genes in our study that showed significant geographic differentiation in response to temperature were also identified, including tim (circadian rhythms gene), Mur18b (chitin binding gene) and Cyp6a19 (Cytochrome P450 gene). The category of P450 gene family has been shown to be highly differentiated in Australian populations (Turner et al. 2008). For both Northern and Southern populations, we observed a large number of P450 gene family genes that were differentially expressed in response to the temperature treatment. We were especially interested in circadian rhythm genes and we found the genes per and tim, which were differentially expressed in both populations and the timeout gene, which was differentially expressed in the Southern population only. Studies that focus on gene expression plasticity, like Levine et al. (2011), provide a better understanding of what functional role these differentially expressed genes are playing. Levine et al. (2011) identified several genes showing Genotype x Environment interaction

patterns and we found Lectin-galc1 differentially expressed in our Southern population dataset, which is involved in carbohydrate binding. They also identified odorant binding protein genes Obp19a and Obp8a which showed expression plasticity in the temperate population. We observed the same differentially expressed genes in our study, but only in our Southern population. Clinal loci previously identified, including Gpdh (Sezgin 2004), hsr-omega (Anderson et al. 2003), Gdh (Eanes 1999), and Hex-C (Eanes 1999) showed up in our screen for temperature in both populations.

In terms of photoperiod, we identified four genes (Act57B, Act79B, Jupiter, mask) based on GO term enrichment that are associated with the structural component of cytoskeleton. Actin cytoskeleton proteins have been shown to mediate circadian rhythm behavior in *D. melanogaster*. (Ojelade et al. 2014). Eip63E is a gene that was identified in a genomic screen by Kolaczkowski et al. (2011) to be highly differentiated between populations in Australia. This gene was identified to be differentially expressed in Southern populations and is on the list of genes that possess a clinal SNP in its upstream region. The GO function category Eip63E falls under is protein serine/threonine kinase activity. Another gene under the same category and identified as differentially expressed in response to the Photoperiod treatment is shaggy. Notably shaggy is one of the eight main genes involved in the Circadian Clock in Drosophila (Vesala et al. 2012) and has been significantly differentiated in QTL screens of thermally selected lines (Rand et al.2010). Preliminary work looking at DGRP lines selected for a clinal SNP in shaggy indicated that the populations with the SNP more common in the Northern populations showed higher

tolerance to heat knockdown assays (Figure B.1). Thus shaggy is an important candidate gene in understanding the differential response to photoperiod in these populations.

Chromosomal distribution of differentially expressed genes

To test for chromosomal distribution of differentially expressed genes we calculated their number on each chromosome arm of the genome. We observed no over representation of the significantly geographically differentially expressed genes when considering each chromosome arm separately (χ^2 , $P > 0.05$, Table 2.3). *D. melanogaster* has a number of cosmopolitan chromosomes which are common in worldwide populations and show an inverse relationship with increasing latitude. (Kapun et al. 2014). We tested for four of the most common cosmopolitan inversions that are found in *D. melanogaster* and observed the effects within the Northern and Southern population. We did not find an over representation of differentially expressed genes in the inversions compared to the other regions of the chromosome for the temperature treatment (hypergeometric test, $P < 0.05$, Table B.6). This indicates that chromosomal inversions do not have a significant role in the differential expression patterns that we have observed for Northern and Southern populations when subjected to different temperature regimes.

Role of genetic differentiation on the variation in the population specific expression plasticity

We predicted that cis-regulatory control would play a large role in gene expression variation. To test this prediction, we identified highly differentiated SNPs in the upstream region of the differentially expressed genes identified for both populations and each treatment. We used the dataset prepared by Fabian et al. 2012 where they sampled the same populations from Maine and Florida, as in our experiment and generated Fst data for those two populations. We used this database to filter out highly differentiated SNPs in the 1kb upstream region of genes and compared that dataset to our list of differentially expressed genes. We found that >50% of the genes that we had identified in our RNA screen possessed a highly differentiated SNP in this upstream region. We tested for enrichment in the list of genes, which had an upstream SNP present for both Northern and Southern populations. We found evidence that differentially expressed genes in both populations were significantly enriched for SNPs in the upstream region (Table 2.4). Based on this filtering method we were able to reduce the number of differentially expressed genes in the temperature x photoperiod treatment: 9 out of 14 genes contained an upstream SNP in the Northern population and only 1 out of 2 genes in the Southern population that had a significantly differentiated upstream SNP (Table B.1). Comparison of known transcription factors with our gene lists with an upstream SNP, indicated that 28 out of 454 genes in the Northern population and 47 out of 958 genes in the Southern population belonged to that category (Table B 2.7).

2.5 Discussion

Our study uses two *D. melanogaster* populations, from the Northern and Southern regions of the eastern United States, to demonstrate that there is a differential response in expression plasticity between flies of discrete geographical origin who were exposed to distinct environmental treatments—temperature and photoperiod. Exposure to extreme temperatures caused high expression plasticity in both the populations, whereas the transcriptomic response to the photoperiod treatment was only observed in the Southern populations based on our cutoff values. These patterns of differential expression validate the genomic differences that have been previously identified in these same populations (Turner et al. 2008, Fabian et al. 2012). Beyond the overall patterns of differential expression, it is crucial to understand the role of the genes responsible for these patterns and how the populations carrying these genes are responding to differences in environmental cues. Differential patterns of expression in spite of high rates of migration in North American *D. melanogaster* (Coyne and Milstead 1987, Berry and Kreitman 1993) suggest that spatially varying selection may be acting on these populations. The recent colonization history of the North American *D. melanogaster* population (David and Capy 1988) further indicates selection on standing variation driving the expression level differences (Zhao et al. 2015).

We observed significant differences in the number of genes showing differential expression patterns between both Northern and Southern populations. The Southern population showed higher expression plasticity in both the temperature and photoperiod treatment conditions. A similar pattern of higher expression plasticity in Southern populations has also been observed for populations placed under different temperature

regimes during development (Zhao et al. 2015). We had previously tested the phenotypic stress response of these two populations under the same experimental conditions for temperature and photoperiod and had observed increased plasticity in stress phenotypes the Northern region populations. The patterns of expression indicate a canalized response for gene expression in these populations derived from temperate regions. There is evidence to suggest that natural selection may preserve canalized expression at the genome level in populations that are under stressful environments to maintain plasticity at the phenotype level (Shaw et al. 2014). Killifish that are exposed to changes in salinity show changes their gill morphology and a reduced gene expression change compared to the control sample (Shaw et al.2014). Similar to the Killifish, the Northern fly population in our previous phenotypic study shows higher stress tolerance and increased phenotypic plasticity, while in this complimentary genotypic study, the same population also exhibits patterns of reduced gene expression. This result is different from the hypothesis that in temperate environments, selection may favor genotypes associated with higher phenotypic plasticity (Gibert et al. 2004). Our experiment was not directly testing for genotypes associated with several stress phenotypes that are plastic. To investigate the genotypes associated with phenotypic plasticity we would require more in-depth functional and gene network analysis work in the future.

Within the context of temperature, the differentially expressed genes show high percentages of enzyme activity and binding gene categories, in terms of functional annotation enrichment. Most of the gene enrichment categories were enriched for metabolic function, a category that shows clinal variation in North America (Eanes 1999,

De Jong and Bochdanovits 2003). When comparing the gene categories enriched for hot versus cold treatment for both Northern and Southern populations, most of the enzyme activity gene categories are enriched in the cold exposure treatment, while the binding categories are enriched in the hot temperature treatment. Other studies of transcriptome levels also see an enrichment of binding gene categories within Drosophilid species that have been exposed to high temperatures (Riveron et al. 2013, Uy et al. 2015). Similarly, single and prolonged exposure to cold treatment causes a down regulation in binding category genes and an up regulation of catalytic activity in *D. melanogaster* (Zhang et al. 2011). Catalytic activity genes are also observed to be down regulated when exposed to heat stress (Sorensen et al. 2005) following the same pattern we observe in our GO term enrichment.

Studies that have identified geographic differentiation in populations of *D. melanogaster* in North America and Australia are especially useful for comparisons to understand the general patterns and specific differentiated genes in these populations. Identifying SNPs through genome sequencing in the coding and non-coding regions provides a dataset that can be used for comparison between studies. We observed an enrichment of upstream SNPs in the differentially expressed genes indicating the role of cis-regulatory transcriptional control of the expression patterns. There is growing evidence to suggest that cis-regulatory mutations play a key role in driving phenotypic evolution (Stern and Orgogozo 2008, Wray 2007). Our list of differentially expressed candidate genes with clinal SNPs in the upstream region provide a valuable dataset of genes that

could be involved in the distinct patterns of expression plasticity and phenotypic differences that we observe in populations of *D. melanogaster* in North America.

Our study exhibits the role of short term exposure to environmental variables on gene expression plasticity in *D. melanogaster*. We have identified differential patterns of gene expression that are specific to the geographic origin of the population. By exposing Northern and Southern populations to distinct and interacting photoperiod and temperature regimes and measuring their gene expression, we have gained valuable insights on what genes are involved in transcriptional modulation in response to conditions that these populations are experiencing in nature. By studying the modules of gene expression and their functional basis we will have a better understanding of how these gene expression changes are affecting fitness in the environment.

Table 2.1: North vs South differential expression for temperature and photoperiod

	North	Common	South
Temperature	434	2359	2518
Photoperiod	0	0	367
Temperature x Photoperiod	14	0	2

Table 2.2: North vs South differential expression based on the temperature and photoperiod treatments – Hot, Cold, Long day, Short day

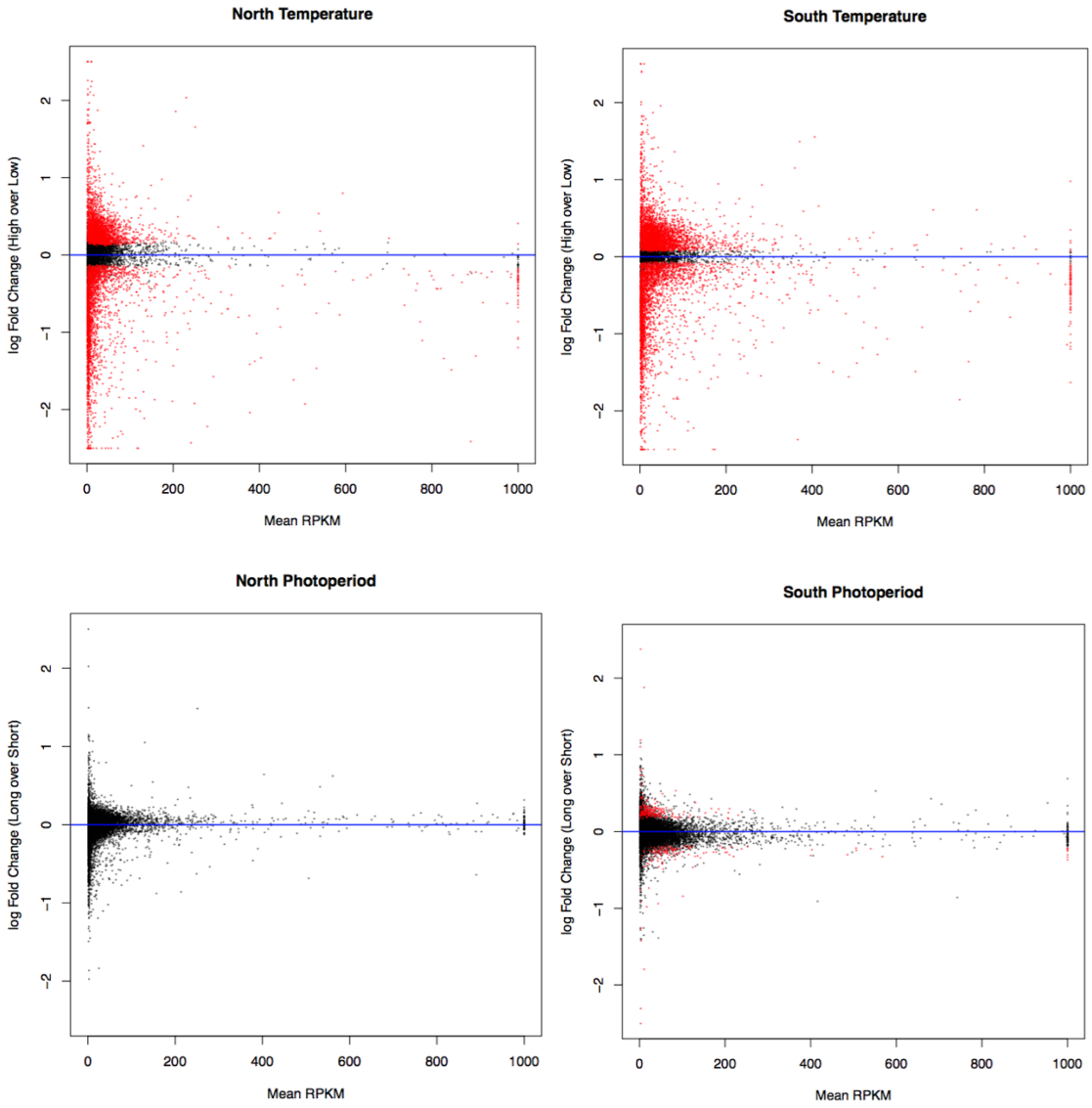
Population	Treatment condition	No of genes upregulated	No of genes not showing DE
North	29°C	1439	2994
North	14°C	1555	2994
South	29°C	2695	2742
South	14°C	2452	2742
South	15 L : 9 D	139	7551
South	9L : 15 D	199	7551

Table 2.3: Chromosomal distribution of geographically expressed genes

Chromosome	No. of genes	No. of genes expressed	No. of genes North	p value	No. of genes South	p value
U and 4	163	58	23	0.934	27	0.202
2L	3288	1408	528	0.938	909	0.877
2R	3270	1649	661	0.136	1041	0.674
3L	3135	1506	565	0.942	981	0.685
3R	3797	1952	721	0.614	1286	0.394
X	2482	1306	471	0.387	808	0.396
Total	16135	7879	2969		5052	

Table 2.4: Upstream SNPs in the differentially expressed genes and enrichment p-values

Population	Treatment	Number of genes differentially expressed	Number of genes differentially expressed with an upstream SNP	Enrichment p-value
North only	Temperature	434	182	<0.0001
Common	Temperature	2359	1004	<0.0001
South only	Temperature	2528	958	<0.0001
South	Photoperiod	367	178	<0.0001
North	Temperature x Photoperiod	14	9	0.080
South	Temperature x Photoperiod	2	1	0.631



Figures 1.1 Log2 fold change of expression levels versus mean normalized RPKM values in North and South populations for temperature and photoperiod.Genes that are significantly differentially expressed (FDR<0.3) in North and South are shown in red. The expression levels are normalized by library sizes. North photoperiod did not have any differentially expressed genes

CONCLUSION

This dissertation demonstrates that (1) short term exposure of adults to various temperature and photoperiod regimes has a pronounced and predictable effect on multiple aspects of fitness, (2) geographically disparate populations exhibit distinct patterns of plasticity in their phenotypic response to stressful conditions and in their gene expression plasticity, and (3) these patterns of differential plasticity are consistent with local adaptation to thermal regime. Together, our results suggest that the short term, physiological acclimation to temperature and photoperiod is modulated by natural selection and may represent an important component in the suite of traits underlying adaptation to the environmental heterogeneity to which this species is exposed.

Physiological acclimation is well described in *D. melanogaster* (Hoffman et al. 2002) and its effect on subsequent traits is also well documented. Heat shock response (Morrison and Milkman 1978, Loeschcke et al. 1994) and chill coma recovery (Gibert et al. 2001, Hallas et al. 2002) are some of the classic examples of thermal plasticity in *D. melanogaster*. I have demonstrated that populations that have been exposed to different climate regimes, have a differential response to environmental heterogeneity. In addition to the laboratory, we observed differential response plasticity in complimentary field experiments, making our results ecologically relevant and enabling better predictions of how organisms behave outside the lab and in the natural world. Interestingly, the differential gene expression plasticity that we found for the two populations is enriched for SNPs in the upstream region. This supports our hypothesis that adult response

plasticity is cis-regulated and indicates that these regions could be under selective pressure from the environment to maintain fitness.

My dissertation helps refine our understanding of the overwhelming genomic variation in natural populations of *D. melanogaster* by providing evidence that this variation is, at least in part, associated gene expression plasticity and performance in response to environmental heterogeneity. Changes in temperature and photoperiod, the environmental cues used in my experiment as proxies for climatic variation, produced a predictable response in the adult life stage of the experimental flies over a short period of time. This suggests that short term response plasticity could be an important, yet often overlooked, component of local adaptation in response to a changing environment.

My dissertation makes several unique contributions to the study of climatic adaptation and the role of phenotypic plasticity. There is not a single paper, to my knowledge, that looks at gene expression plasticity in response to photoperiod using more than one natural population of *D. melanogaster*. The results from my genomic experiments clearly show that the Southern population shows a strong effect of photoperiod, whereas the Northern population shows no effect of photoperiod. These results support for a long standing hypothesis that Southern populations are much more sensitive to day length variation than their Northern counterparts (Tauber et al. 1986). In this way, my recent genomic analysis is able shed light on important established phenotypic observations in the field.

In conclusion, this work details the importance of short term response plasticity as a mechanism of local adaptation to environmental heterogeneity in natural populations of *Drosophila melanogaster*. Studying phenotypic response in both lab and field environments provides a better understanding of what cues are driving the observed response patterns. These phenotypic studies are complimented by studies of differential gene expression plasticity, which have provided a list of candidate genes that are potentially under spatially varying selection due to the environmental fluctuations to which these natural populations are exposed.

APPENDIX*

APPENDIX A: Chapter One

Table A.1: Temperature and photoperiod data from field experiment from 2012-2014

Table A.2: Statistical results for difference between northern and southern regions at different treatment conditions

APPENDIX B: Chapter Two

Table B.1: Gene list of differentially expressed genes
Table B.2: Gene list of differentially expressed genes based on the temperature and photoperiod treatments
Table B.3: GO term enrichment for differentially expressed genes
Table B.4: GO term enrichment for differentially expressed genes based on the temperature and photoperiod treatments
Table B.5: Comparison of differentially expressed genes with candidate genes from Zhao et al. (2015)
Table B.6: Differentially expressed genes in regions spanned by cosmopolitan inversions
Table B.7: Comparison of differentially expressed genes with known list of transcription factor genes
Table B.8: DAVID analysis for GO category enrichment in genes which are uniquely differentially expressed in the north and south populations
Table B.9: Molecular function of genes that are differentially expressed in the temperature x photoperiod treatment
Table B.10: Box plots of the 32 cDNA libraries
Table B.11: Comparison of differentially expressed genes for FDR<0.1, 0.3 and 0.5
Table B.12: List of uniquely differential expressed genes upregulated in hot and cold treatment conditions
Table B.13: Number of uniquely differential expressed genes upregulated in hot and cold treatment conditions
Figure B.1: Heat knockdown assay for two alleles of shaggy gene generated from DGRP lines

*(Submitted to the online repository)

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Chapter One

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