The Genetic Basis of Phenotypic Plasticity in Natural Populations of Drosophila

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Abstract
Environment-dependent phenotypic expression, also known as phenotypic plasticity is exhibited to some degree by all organisms. Natural selection can act on the ability to respond to the environment allowing individuals to maintain fitness across heterogeneous environments. However, phenotypic plasticity can also potentially slow the rate of adaptive evolution within a population or result in maladaptive phenotypes. Despite the widespread occurrence and consequence for adaptive evolution, the genetic architecture and specific molecular variants that underlie phenotypic plasticity remain largely unknown. To evaluate patterns of plasticity and the genes that mediate the plastic response this work utilizes Drosophila melanogaster and its close sister specie Drosophila simulans. Individual lines collected from natural populations of D. melanogaster and D. simulans have previously been shown to exhibit phenotypic plasticity for several traits in response to changes in temperature and nutrition. However, these studies do not address patterns of plasticity across heterogeneous environments. To establish that the strength of the plastic response varies within and among natural populations isofemale lines of D. melanogaster and D. simulans were collected from three locations along the east coast and exposed to various larval rearing environment. The geographic pattern in the strength of the plastic response is only present in some traits and absent in others, which highlights the modular nature of phenotypic plasticity. To identify a gene that is able to modulate plasticity across several life history traits this work takes advantage of a candidate gene approach. A previously identified genetic polymorphism in the couch potato (cpo) gene in D. melanogaster mediates the propensity to diapause is shown in this work to affect the individual's ability to respond plastically across several life history traits. The patterns observed in the investigation of cpo parallel the patterns of plasticity observed in natural populations. Thus, polymorphism in cpo gene may play an important role in the mediation of plasticity in natural populations. These findings provide insight into plasticity within natural populations and the genes that underlie the strength of the plastic response.

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THE GENETIC BASIS OF PHENOTYPIC PLASTICITY IN NATURAL POPULATIONS OF DROSOPHILA

Katherine Rogan O’Brien

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Biology

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THE GENETIC BASIS OF PHENOTYPIC PLASTICITY IN NATURAL POPULATIONS OF *DROSOPHILA*

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I dedicate this dissertation to Katty Rogan who taught me to strive, to Kay Paltsios who inspired my love of science and to Nancy O’Brien who was always my fiercest advocate.
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Environment-dependent phenotypic expression, also known as phenotypic plasticity is exhibited to some degree by all organisms. Natural selection can act on the ability to respond to the environment allowing individuals to maintain fitness across heterogeneous environments. However, phenotypic plasticity can also potentially slow the rate of adaptive evolution within a population or result in maladaptive phenotypes. Despite the widespread occurrence and consequence for adaptive evolution, the genetic architecture and specific molecular variants that underlie phenotypic plasticity remain largely unknown. To evaluate patterns of plasticity and the genes that mediate the plastic response this work utilizes *Drosophila melanogaster* and its close sister specie *Drosophila simulans*. Individual lines collected from natural populations of *D. melanogaster* and *D. simulans* have previously been shown to exhibit phenotypic plasticity for several traits in response to changes in temperature and nutrition. However, these studies do not address patterns of plasticity across heterogeneous environments. To establish that the strength of the plastic response varies within and among natural
populations isofemale lines of *D. melanogaster* and *D. simulans* were collected from three locations along the east coast and exposed to various larval rearing environment. The geographic pattern in the strength of the plastic response is only present in some traits and absent in others, which highlights the modular nature of phenotypic plasticity. To identify a gene that is able to modulate plasticity across several life history traits this work takes advantage of a candidate gene approach. A previously identified genetic polymorphism in the *couch potato* (*cpo*) gene in *D. melanogaster* mediates the propensity to diapause is shown in this work to affect the individual’s ability to respond plastically across several life history traits. The patterns observed in the investigation of *cpo* parallel the patterns of plasticity observed in natural populations. Thus, polymorphism in *cpo* gene may play an important role in the mediation of plasticity in natural populations. These findings provide insight into plasticity within natural populations and the genes that underlie the strength of the plastic response.
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CHAPTER 1

INTRODUCTION TO THE GENETIC BASIS OF PHENOTYPIC PLASTICITY

Naturalists have been aware that the environment affects the phenotype of an individual for longer than we have had a working concept of genetics or evolution. All organisms display the ability to respond to the environment to some degree. This environmentally sensitive production of alternative phenotypes from a single genotype is referred to as phenotypic plasticity (Bradshaw 1965; West-Eberhard 2003). Despite being a common phenomenon we still know very little about the genetic basis of plasticity or how it varies in natural populations. Theories developed in the past twenty years have begun to address our understanding of the origins (West-Eberhard 1989), costs (DeWitt et al. 1998; Relyea 2002), maintenance ( Getty 1996; Pigliucci 2005) and adaptive nature (Via et al. 1995; Ghalambor et al. 2007) of plasticity. However, many of these predictions about the distribution and genetic basis of plastic ability remain largely untested (Scheiner and Holt 2012). This results in a classic “black box” problem where the input and output are known while the mechanism remains a mystery. Environmental inputs are given, and the resulting trait is returned, yet, some individuals respond to the environment more strongly than others. It is genetic differences between those individuals that control how an individual perceives the environment and gene expression that ultimately produces the phenotypes. The genetic variation and mechanisms that result in the perception and response are responsible for regulating the strength of reaction to the
environment yet many remain unknown.

The very nature of phenotypic plasticity is challenging to address because it is not the value of a trait but rather the variation in the trait among environments that is of interest. Classic genetic techniques, which focus on a particular trait in a constant environment, are not designed to detect this sort of variation. Elucidating the genes involved is further complicated by the assumption that plasticity results from the interaction of many genes not a single gene (Schlichting 1986; West-Eberhard 2003). Despite these challenges, progress has been made by using candidate gene approach as well as utilizing techniques that screen for genes underling complex traits such as quantitative trait locus (QTL) mapping.

The Genetic Basis of Plasticity

Most of the progress in identifying the genetic variants that mediate the plastic response has been accomplished mostly through the use of the model organisms *Drosophila melanogaster* and *Arabidopsis* since candidate genes, quantitative trait loci (QTL) mapping and association studies are most effective in these organisms. Research over the past 20 years has commenced to build a list of genes and gene networks that modulate the plastic response. Not surprisingly, most of the genes and pathways that have been identified as having the ability to modulate plasticity are associated with some aspect of the environment sensing. This pattern holds true for both animal models and
plant models for plasticity. In *Drosophila melanogaster* genes in the insulin signaling has been linked to the phenotypic plasticity specifically through the genes *dare* (Bergland et al. 2008), *forkhead transcription factor* (FOXO; Libert et al. 2007; Tang et al. 2011) InR (Green and Extavour 2014) and CHICO (Naganos et al. 2012). *Arabidopsis* as well as other plant systems the genes controlling flowering time that are connected to sensing far-red wavelengths (*FRIGIDA* or *FRI*; Johanson et al. 2000 and *FLOWERING LOCOUS C* or *FLC*; Michaels and Amasino 2001). However, it is rare that these molecular variants are discussed in the context of natural populations.

Some notable exception is a series of work done on shade avoidance in natural populations (Schmitt et al. 1999; Schmitt et al. 2003) and clinal patterns in sunflowers. Also the work on flowering time has been informed by natural variation (Mitchell-Olds and Schmitt 2006) as well as the use of natural populations to derive a variant of *FRIGIDA* that varies between populations (Kuittinen et al. 2008). This work has been recently generalized to other plant system such as the sunflower. Work in the sunflower examined how genes function in a gene network to mediate plasticity. Specifically, gene expression in the light sensing and gibberellin pathways was measured along a latitudinal cline to examine their effects on plasticity in flowering time. Multiple genes within these networks were associated with mediating the plastic response, suggesting that modularity is very important in the process of evolving plasticity (Blackman et al. 2011). This work on natural populations builds on our knowledge of the genetics of plasticity while discussing plasticity in adaptive and population-based context.
The following chapters

In the following chapters I present an examination of natural variation in phenotypic plasticity across several life history traits in the model organism *Drosophila melanogaster*. The aim of this work is to first measure plasticity in natural population and then to identify a genetic variant that mediates the plastic response. To measure plasticity in a natural context this work utilizes collection of isofemale lines from orchards made by the members of Dr. Paul Schmidt lab including myself. The majority of plasticity work previously done using fruit flies has focused on variation in rearing temperature.

Although this is an extremely relevant environmental variable for an ectothermic animal, several other variables that vary in natural populations remain under explored. This work focuses on variation in the fruit type available to larvae and measures the effect in the adult phenotypes. Flies in orchard populations lay eggs on multiple different types of fruit each of which presents its own environmental challenges to developing larva. In these studies we used apples, which present a stable environment for larval development and strawberries, which are more ephemeral. To reduce the effects of nutritional differences, fly media was made iso-caloric for sugar content (described in the following chapter) although still differed in micronutrients. In the second chapter, of this work photoperiod was also manipulated to evoke a plastic response. Two photoperiods were chosen a long day period (15L:9D) and a short day period (9L:15D), which reflect the extremes in photoperiod experienced in the mid-Atlantic region (39.95° N). Photoperiod is thought to be a reliable cue in the detection of seasonal changes. Using
flies derived from natural populations while varying food and photoperiod both important
cOMPONENTS OF THE ENVIRONMENT EXPERIENCED BY LARVA this work is able to address
plasticity in a natural context.

The chapters Two and Three are experimental summaries written as independent
manuscripts. Chapter Four concludes the dissertation with a summary of the results from
both experiments and suggests for future directions. Chapter Two establishes that natural
populations have different abilities to respond plastically to their environment. This
chapter emphasizes the importance of how plasticity is measured while comparing clines
in the strength of the plastic response for the two sister taxa Drosophila melanogaster and
Drosophila simulans. Both species have adapted to many novel environments, ranging
from tropical climates to temperate zones (David and Capy 1988; Irvin et al. 1998).
Despite their overlapping ranges and similar appearance these sister species have distinct
ecology and behavior (Parsons 1975). Natural populations of both species collected from
different locations along a cline offer a rare opportunity to conduct a detailed
investigation of plasticity in two closely related organisms. Chapter Three uses a
candidate gene approach to investigate a naturally occurring allelic change in the gene
couch potato affects the ability to respond plastically across several life history traits in
Drosophila melanogaster. This is the first evidence that separate alleles can differentially
mediate the plastic response across multiple traits. The conclusion discusses the
connections between these two experiments as well as offers insights into how best to
identify and characterize other genes that mediate plasticity.
CHAPTER 2

PATTERNS OF NATURAL VARIATION IN THE PLASTIC RESPONSE FOR TWO SPECIES OF DROSOPHILA

Abstract

Plastic responses are thought to be evolutionarily favored under specific conditions, yet many theoretical predictions about the distribution of these responses remain untested. Theory predicts populations that encounter more environmental variation should have a higher incidence of plasticity when compared to populations found in more stable environments. Expanding on this idea panmictic species with a larger range should be more plastic when compared to closely related species with a smaller range. To test these predictions plasticity was measured in two species, Drosophila melanogaster and its closest relative, Drosophila simulans, collected from three locations along a latitudinal cline. Plasticity in three life history traits, development time, body size and lipid content were all assessed by manipulating larval rearing media and photoperiod both chosen to represent cues in experienced by natural populations. The strength of the plastic response in larval development time demonstrated a pronounced cline in both species. However, neither body size nor lipid content demonstrated a clinal signature though both traits showed differences between populations and a high degree of responsiveness. These data demonstrate that patterns of natural variation in plasticity are trait specific. Furthermore, the parallelism in these responses between the sister species suggests that similar environmental pressures are driving selection for these patterns.
Introduction

Every organism encounters variation in environmental conditions across temporal and spatial scales throughout its lifetime. This variation changes the fitness landscape such that high fitness traits in one environment may become neutral or unfit in a second environment. Phenotypic plasticity is one mechanism that has evolved to maintain fitness across variable selection landscapes (Bradshaw 1965; Stearns 1989; West-Eberhard 2003). This is not to suggest that the evolution and maintenance of plasticity in populations is always favored because in some cases plasticity is clearly maladaptive (Donohue et al. 2000). The potential for environmental mismatch can select against the evolution and maintenance of plasticity within a population (Berrigan and Scheiner 2004). Based on these theoretical models we would expect that plasticity and the strength of the plastic response to vary between populations. Variation in plastic ability has been documented on small local scales (Wilken 1977), between closely related species (MacDonald et al 1989), across different fitness landscapes (Donohue et al. 2001) and along latitudinal clines (Blackman et al. 2011). These differences between populations offer the ability to address how changes in environmental factors could affect the evolution of the plastic response. Clines in environmental parameters such as, temperature, photoperiod, food availability and competitive regime offer a unique natural experiment to test for a relationship between environmental factors and the strength of the plastic response.

*Drosophila melanogaster* and its sister species *Drosophila simulans* presented an opportunity to evaluate plasticity in natural population across a cline. Both species are human commensals found in natural populations all over the world since expanding from
its original range in sub-Saharan Africa. *Drosophila* have adapted to many novel environments, ranging from tropical climates to temperate zones since their arrival on the North American content a few hundred years ago (David and Capy 1988; Irvin et al. 1998). The two species have overlapping ranges but a very different ecology and behavior. Notably, *D. melanogaster* can survive the temperate winter, which allows for permanent populations along the entire east coast of the United States. Alternatively, *D. simulans* cannot survive the winter and must re-colonize northern climates every summer (Parsons 1975). This wide environmental range and diverse ecology makes the fruit fly sister species an excellent system for addressing hypothesis in evolutionary ecology (Capy and Gibert 2004). Variable natural populations along a latitudinal cline offer an opportunity to conduct a detailed investigation of an organism for patterns of phenotypic plasticity in a natural context.

The challenges with understanding patterns of plasticity in natural populations are complicated further by deciding what aspect of plasticity should be quantified. The most widely used approach to measure plasticity is the examination of genotype by environment interactions through the use of analysis of variance (ANOVA). Plasticity is considered to be present when the different genotypes have different mean trait values in between environments. Furthermore a significant genotype-by-environment interaction term results from the genotypes demonstrating different abilities to respond (Schlichting 1986; Scheiner 1993). However, since ANOVA is measuring trait means and thus cannot capture aspects of plasticity that are associated with the individuals reaction norms. There is a growing interest in expanding the statistics to measure plasticity such that theories regarding the strength of the plastic response may be tested (DeWitt et al. 1998). Resent
work by Kingsolver and colleges used a novel method for using reaction norms to address developmental time in response to temperature. A common measure of plasticity is to simply look at the differences between the means across environments. However, the effects of temperature are so great they can mask the variation between different genotypes. To address the problem Kingsolver et al. analyzed the residuals of the mean difference of the genotype minus the reaction at the temperature of interest. This vertical shift value for the set was the measure of plasticity allowing a more in-depth analysis of the patterns (Kingsolver et al. 2014).

We introduce a different approach based on calculating an effect size for each genotype and performing a meta-analysis. Similar to Kingsolver et al., this approach is focused on being able to measure plasticity within a genotype. The advantage to using effect size is that it is an unbiased measure and may be used on multiple environments or traits at the same time. For either of these techniques to be successful in evaluating plasticity among multiple populations, a large sample of lines needs to be acquired to quantify the reaction.

Life history traits are characteristically complex, polygenic and can be particularly sensitive to environmental perturbations such as changes in temperature, nutrition, competition and predation regime (Roff 2002). As such, they provide an excellent set of traits that should respond plastically and be subject to natural selection. For this study the three traits measured (larval development time, adult body size and adult lipid content) represent and integrative responses to larval environment. Development time is environmentally sensitive and one of the most important aspects of life history with fitness consequences (Santos et al. 1994; Roff 2002; Kingsolver and
Huey 2008). Body size and lipid content are two measures of downstream metabolic outcomes from food acquisition and allocation during the larval stages and metamorphosis in the puparium (Boggs 2009). Both body size and development time have been shown to affect fitness in D. melanogaster and to respond plasticly with pervasive G X E interactions in response to temperature (James et al. 1997) and density (Santos et al. 1994). These three phenotypes though related provide three responses to larval environment to address patterns of plasticity in natural populations.

If more environmental heterogeneity simply leads to a great plastic response we would expect three patterns to emerge along latitudinal clines (Blackman et al. 2011). First, flies from the southern populations should be more sensitivity to changes in rearing media. In the south the growing season is longer making a wider variety of fruit available as larval rearing environments. Secondly, flies from northern populations should be more sensitive to difference in photoperiod because populations in the northern latitudes experience greater variance in day length. Finally, D. melanogaster should be more plastic than D. simulans because its populations have a larger permanent range and could have evolved the ability to deal with a larger range of seasonal environments. If pressure from environmental heterogeneity must be combined with other selection factors we would expect that the ability to respond but populations are still maintaining plasticity then we would expect to see flies that are responsive but without the clinal signature described above. To address these predictions regarding the maintenance of plasticity and its relationship to environmental variation, we measured plasticity across each trait in two Drosophila species collected from three locations along the east coast: Maine, Pennsylvania, and Florida.
Materials and Methods

Fly Stocks and Culture

*Drosophila melanogaster* and *Drosophila simulans* females were collected from four locations, Fruit and Spice Park (Homestead, FL 25.53°N: -80.49°W), Lawson Peach Shed (Morven, GA 30.94°N: -83.49°W) Linvilla Orchards (Media, PA 39.88°N: -75.41°W), Westward Orchards (Harvard, MA 42.49°N: -71.56°W), and Rocky Ridge Orchards (Bowdoin, ME 44.03°N: -69.95°W) from 2008 to 2012 to establish isofemale lines. In addition, isofemale lines from Linvilla were collected at two seasonal time points, once in May and again in November of the same year, 2012. The male offspring were examined for specie determinations. These lines were maintained at room temperature on cornmeal-molasses media with 2% yeast by volume for no less than four generations to remove maternal and grand-material effects and some lines were sampled after up to two years of lab culture (Table 1).

Life History Trait Measurements

In these experiments two aspects of the rearing environment food type were manipulated. Rearing media was iso-caloric and based on either apple or strawberry mash. Both types of media contained 1000 ml deionized water, 28.0 g agar, 67.7 g
nutritional yeast and 4.97 g methylparaben dissolved in 99.4 mL 95% ethanol.

Strawberry and apple have different calories per g with apples being more calorically dense to correct for this the food was standardized to 680 kilocalories of fruit per 1000 ml water. To make the fruit mixtures iso-caloric, 2122.8 g of strawberries or 1306.6 g of cored apple were homogenized in a blender and deionized water was added to bring the total volume of fruit add to 2215.6 ml. The agar solution was heated to a rapid boil then the fruit mixture and yeast were added. This was then cooked for 60 min at a low boil, cooled to 55°C, methylparaben was added and then poured. This recipe yields foods that are similar in sugar and yeast content but with different fruit bases. Photoperiod was also manipulated shot day (9L:15D) and long day (15L:9D). This design yields four different environments to elicit a phenotypic response: 1) apple short day, 2) apple long day, 3) strawberry short day, and 4) strawberry long day. All experimental flies were reared at 25°C in Percival I36VL incubators in cambers were the photoperiod could be set to long days or short days. Temperature in the chambers remained the same despite differences in the light cycle by the continual circulation of air with a fan and s bend vents. Three components of life history (development time, body size and lipid content) were measured in the sequence presented below. Each line was measured twice in each of the four experimental treatments yielding 8 measurements per line for each phenotype.

Phenotypic assays were initiated by placing grape juice-agar plates with yeast paste (active yeast and water) were placed in the population cages overnight to collect a cohort of eggs. To ensure uniform density eggs were individually transferred to a vial containing experimental food medium at a low density of 20 eggs per vial. Both the time to pupation and the time to eclosion were measured for development time. Larval stage
was tracked daily until pupation; after the formation of pupae, the individual pupae were numbered and tracked until eclosion. Pupation and eclosion were assessed at 4-hour intervals until the last viable pupa eclosed. Once eclosed, adults remained in their rearing vial at 25°C in the rearing photoperiod in mixed sex groups until age 1-5 days. Once the last viable pupa eclosed the adults were moved to microcentrifuge tubes under anaesthetic and stored at -20°C.

Adults were stored at -20°C until they were sorted into same sex groups to be weighed. Samples of either 5 males or 4 females were dried at 60°C for a period of 56±3 hours to obtain the initial dry-weight measurement using a Sartorius R160P balance and weighed to the nearest 1x10⁻³ g. Total body lipid content was measured using ether extraction (Robinson et al. 2000). The samples were then left for 24 hours in 0.5 ml of diethyl ether to extract lipids. Extracted flies were then dried for 24 hours at room temperature and weighed again to calculate the total body lipid content as the difference between the initial dry-weight and the weight following the ether extraction.

Statistical analysis

For the purpose of this study lines from Fruit and Spice Park in Florida and Lawson Peach Shed in Georgia were pooled to represent populations of southern origin. The lines from Rocky Ridge Orchards in Maine and Westward Orchard in Massachusetts were pooled to represent populations of northern origin. The sample from Linvilla Orchard in Pennsylvania represents a midpoint between the southern and northern regions and will be referred to as the mid-Atlantic origin. For geographical analysis the
collections from the two time points were pooled however for analyses in respect to season they were analyzed separately. Not every egg survived to eclosion. However, total survivorship was statistically equivalent across media types, photoperiod, and between genotypes across the populations. All statistical analyses were run using JMP v.9.0.0 (SAS Institute, Cary, NC).

All traits were assessed using a three-way ANOVA media type x population x photoperiod with isofemale lines nested within population and experimental treatment. Development was assessed in two different ways, which were time spent as a larva and the time in the puparium. Mass and lipid content were log transformed prior to analysis; this yielded results that were qualitatively identical to analysis using the residuals resulting from the regression of body size and lipid content (not shown).

For each isofemale line we calculated effect size (Hedges’ g) as a measure unbiased estimator of the response as a difference between the mean treatment conditions penalized for high variation or low samples size (Gurevitch and Hedges 2001). Hedges’ g was calculated using lines reared on apple as the control group and those reared on strawberry as the experimental group. Furthermore, the absolute difference of the averages was calculated instead of the more traditional difference between the averages. The meta-analyses were conducted using the R studio (version 0.97) and the package ‘MAd’ (version 0.8). The effect sizes for each isofemale line were pooled according to the random effects model, and differences between subgroups of studies tested using the mixed effects model. The subgroups of interest were a direct comparison between the species and the comparison between the geographic subgroup for each species. As indicators of heterogeneity of pooled effect sizes, we calculated $I^2$, which indicates the
heterogeneity in percentages, and we tested whether the level of heterogeneity was significant using the Q statistic (not shown). Here we were interested in the k estimate and its variation as a measure of the response to the environment for each subgroup.

Results

The drawback to using ANOVA to address patterns in plasticity is that individuals that have opposite reactions to the environment can mask differences in the average effect. That appears to be the case for development time where averages for the reaction norms and the lines which those averages were calculated show a very different picture (compare Figure 1 to Figure 2). Although the averages capture the overall direction of the reaction norms in the range of response, a portion of responding individuals are not represented. From the reaction norms there seems to be potential that the portion responding and the strength of that response are not being accurately addressed yet varies between origins. We will address both these methods in the results by first presenting the results from the ANOVA and then presenting the results from the meta-analysis. Both results from the ANOVA analysis and the meta-analysis to yield a more holistic examination of the patterns observed for the three phenotypes of interest.

Development

Development time was partitioned into two stages: the time spent as a larva and the time spent in the puparium. Both show similar patterns. In the ANOVA for larval D. melanogaster, only the main effects, origin, rearing media and photoperiod were
significant (Table 2). In contrast for larval *D. simulans*, not only the three main effects but also the interaction of origin-by-media and origin-by-photoperiod were significant. In regards to a clinal pattern in the strength of the plastic response, *D. simulans* populations of southern origin appear to be more responsive in larval development time across the different environmental combinations (Figure 1). Populations from the north were the least responsive and mid-Atlantic are an intermediate. There was no clinal pattern for strength of plasticity for *D. melanogaster* in either of the developmental stages.

The meta-analysis disagrees with the ANOVA on many points outside of the generalization that fruit flies responded to the environmental treatments and origin was a driver of this response. Using a meta-analysis approach captures the differences between the species with *D. melanogaster* being marginally more responsive across all locations (*D. melanogaster*: $k=4.453\pm0.12$ s.e.; *D. simulans*: $k=3.389\pm0.15$ s.e.). The strength of response for both the species was very similar with a clear clinal signature. The southern flies of both species have a greater response than the northern flies with the mid-Atlantic flies were intermediate (Figure 3). The response to rearing media is stronger than the response to photoperiod. While responsiveness to rearing media was linear, the responsiveness to photoperiod displays a different pattern. *D. melanogaster* from the mid-Atlantic and the south had the same strength of response to photoperiod. Conversely, *D. simulans* from the mid-Atlantic and the north had the same strength of response in regards to photoperiod. This forms a shallower cline in the ability to respond to photoperiod for both species.
In the ANOVA for development time in the puparium for *D. melanogaster*, only the main effects of origin and photoperiod were significant (Table 2). *D. simulans* shows similar however the main effect of media was also significant. Time spent as a pupa responds to changes in photoperiod and media in both species (Figure 1). The overall response to environmental change was small and the strongest difference was that between origins. Furthermore, looking at the main effects and the means there was no difference no clinal pattern in either species.

The results from the meta-analysis are fairly consistent with the ANOVA analysis. The meta-analysis also found that flies of both species responded to media and photoperiod (Figure 3). The importance of origin was diminished with only *D. melanogaster*’s response to media varying between the origins such that the south was less responsive then the other origins. This means that although the trait values between the origins were different their ability to respond to difference in photoperiod and rearing media were relatively equal. From both analysis we can say that pupa responded to differences in environment where *D. melanogaster* showed more sensitivity however this response does not vary with origin. Furthermore, the response of the pupa was overall smaller compared to the ability for the larva to respond especially in regards to the southern origin. This was also clearly seen in the reaction flatter reaction norms.

Body size and lipid content

Body size (dry mass) and lipid content (total body lipid content) are discussed together because both measures represent downstream metabolic outcomes resulting from
food acquisition and allocation during development. As expected, males were significantly smaller and were characterized by reduced lipid content and therefore the sexes were analyzed separately.

Female mass was affected only by origin (Table 2). *D. melanogaster* mid-Atlantic females were significantly larger while, *D. simulans* females from the south were larger. The effect of origin was clinal for *D. simulans* but not *D. melanogaster*. Regardless of experimental treatment southern flies were the biggest, followed by the mid-Alantic and then north (Figure 1). This pattern did not hold when we look at the strength of the response via the meta-Analysis. Although there were differences between the origins and both species showed strong responses to the environment no pattern emerges (Figure 4).

Lipid content of females was only affected only by media in *D. melanogaster* (Table 2). *D. melanogaster* females reared on strawberry food stored more lipid then those reared on apple food regardless of photoperiod or origin. Similarly to female body size, the effect of origin was clinal for *D. simulans* regardless of experimental treatment southern flies are the biggest follow by the mid-Alantic and then north (Figure 1). Again the pattern seen in the trait values does not hold when the strength of the response was examined via meta-analysis. Although there are differences between the origins and both species show strong responses to the environment no definitive pattern emerges (Figure 4).

Mean male mass, unlike female mass, showed different patterns between the species (Table 2). In *D. melanogaster*, only the main effects of origin and media were observed. Similar to *D. melanogaster* females, this effect is driven by the large size of the mid-Atlantic flies. In contrast, *D. simulans* male mass was affected by photoperiod and
the origin-by-photoperiod interaction. Unlike the results from female mass, there is no indication of a clinal pattern by looking at the trait values. However, when considering the differences between the means, males from the south appear to have little response to changes in the environment while the males from the north were much more responsive. The meta-analysis confirms the lack of a clinal signature for *D. melanogaster* but showed a clinal pattern for the *D. simulans*. There appears to be a greater ability to respond in the north with a reduced response in the south (Figure 4). Interestingly, these were the least responsive flies in the entire study with strength of response of only 3.4. Both species show strong responses to the environment only *D. species* shows a clinal pattern.

Although the small sample size for *D. simulans* males compared to the sample size *D. melanogaster* may have driven this result.

Male lipid content showed different patterns between the species (Table 2). In *D. melanogaster*, effects of origin and media as well as the origin-by-media interaction were observed. Similar to the females, males reared on apple food stored more lipid, and males from the mid-Atlantic region stored more lipid then those from other origins. *D. simulans* male lipid storage was affected by origin and the origin-by-photoperiod interaction. Unlike the results from female mass, there is no indication of a clinal pattern, however southern males tend to store more lipid then their counterparts. Unlike male mass, there was no indication of a pattern in the difference to respond for either species. This result was confirmed with the meta-analysis since even though there are differences between the origins and both species show strong responses to the environment no definitive pattern emerges (Figure 4). Overall, both males and females of the sister taxa respond to
media and photoperiod there is no pattern that is dictating the strength of response with the exception of body size in male *D. simulans*.

**Discussion**

**Analysis: ANOVA versus effect size**

A common problem in the field of phenotypic plasticity is deciding what to measure. We used ANOVA, the most common approach, side by side with a novel use of meta-analysis to do a line-by-line comparison. The ANOVAs were able to answer questions in regards to average trait values and interactions, which are often used to suggest genotype by environment interactions as a form of plasticity. For most studies the difference between the means can be used to give anecdotal evidence about the relative effect (see Chapter 3) however as the design gets more complicated and there are strong effects of line this may no longer possible. Furthermore, when the sign of the difference between to responsive is opposite they can cancel out each other’s effect. Hedge’s g is a metric designed to measure of effect size in this study we used effect size as a proxy for the strength of the response to an environmental change. By using this statistic we were able to quantify a nonbiased response variable, Hedge’s g, for each line and then analyze that response via a meta-analysis. Given the multiple factors, two species, three phenotypes and four environments, used in this study this method allowed us to see patterns that were not always evident by looking at the mean response as well as confirm patterns noticed in the mean response. These two methods, ANOVA and meta-analysis
each added to the ability to recognize and evaluate the patterns discussed bellow in a manor which could not have been accomplished by the standard use of ANOVA.

Natural variation for plasticity

Theory predicts that environmental heterogeneity plays a critical role in selecting for and maintaining phenotypic plasticity. Environmental clines are a useful tool for in investigating the role of environmental heterogeneity assuming heterogeneity varies spatially. More varieties of fruit are grown in the southern United States spread over a longer growing season. Under this hypothesis the southern populations should be more responsive to changes in rearing media due to the greater food resource diversity at southern latitudes. Similarly, northern flies may be more responsive to photoperiodic cues due to the more extreme photoperiods of the northern latitudes. Furthermore, we predicted that *D. melanogaster* would shower more plasticity then *D. simulans* due to seasonal dynamics, which expose resident populations of *D. melanogaster* to the temperate winter. To assess these hypotheses we focused on the measure of strength of the response over the trait values. In the case of development time these hypothesis were in part supported although northern populations were not responsive to photoperiod. However, in regards to body size and lipid content our data present a more complicated trait specific and sex specific interpretation of plasticity in natural population.

Larval development time showed the clinal pattern that we predicted for response to changes in rearing medium in both species. As potential food types became more
diverse and the growing season longer flies of both species responded plastically to changes in fruit type. Flies from all origins tended toward faster development on strawberry food, which is the more ephemeral of the two food sources as strawberries completely rot within weeks of ripening. Also under long days development time was decreased. This may be a result of increased completion in the summer during population expansion simply a byproduct of more active time in which to ingest food. The cline in response to photoperiod was more shallow then the response to rearing media. Interestingly, the southern flies of both species were also the most responsive to these changes in food and photoperiod. They also displayed the greatest range of response accounting for both the slowest and the fastest lines to developed, this range was largest for D. melanogaster. This could be a result of greater genetic diversity but unlikely since no link between outbreeding and plasticity has been established (Via and Lande, 1985). The clinal pattern in the strength of the plastic response suggest the possibility that a plastic response to these environmental cues gives an adaptive edge in a population selected for faster development and more resource competition. However, spatial patterns cannot be considered evidence of adaptive plasticity and a study to measure the fitness consequences in each location is needed (Donohue et al. 2000). While there is no change in trait values, these data do support a latitudinal cline in the strength of the plastic response.

Fast development time showed be selected for so it is curious that development time varies by almost 100 hours in this data. Although this pattern is theorized that development time and body size should be negatively correlated (Roff 2002) this relationship is not commonly observation in insects (Nylin et al. 1993; Nylin and
Gotthard 1998; Scharf et al 2006; Jiménez-Cortés et al. 2011). These data did not show a direct trade off between development time and adult body size in either species. In *Drosophila* work has been done to show that quick development time may be negatively correlated with survivorship and fecundity (Metcalfe and Monaghan 2001) although more research is need in this area.

Patterns in body size and lipid content did not follow the predications and showed no clinal pattern although both phenotypes responded strongly to both environmental cues. The average strength of response was often higher for body size and lipid content then the response of to development time. The exception being that flies of a southern origin were more responsive to medium in regards to development time than they were in regards to lipid content. However, melanogaster males and females from southern origins showed some of the strongest responses recorded in this study. The absence of a clinal signature paired with this high level or plasticity suggests that other selective forces beyond environmental heterogeneity affect the evolution of plasticity in regards to body size and lipid content. Many models that although necessary, environmental heterogeneity alone is often not sufficient for plasticity to evolve (Berrigan and Scheiner 2004). Cost of plasticity may be playing a role in the variation seen in these data. Doubtful in the form of maintenance of the response since the responses is so strong however the cost of producing the trait could also vary between the populations, as could the reliability of food cues. However these data are unable to identify what costs of plasticity could be effecting these populations.

Our results show that plasticity can evolve and be maintained across different traits along a spatial scale. The parallelism in response in larval development time
between two closely relates species, despite different ranges, suggests that strong selection for the strength of the plastic response could be responsible for maintaining these patterns. The modularity of patterns of plasticity between traits that show a plastic response does support the hypothesis that multiple pathways underlie the ability to be plastic in natural populations. However, these data are insufficient for addressing the cost or fitness benefits of plasticity. This data would be needed to make assertions about the adaptive nature in the variation of the strength of responses.
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<th>Second run</th>
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Table 1: lines used in each location with time in lab at assaying. Approximate dates of when assays were run (i.e. First run and Second run) are given in days past collection dates.
Table 2: Summary of ANOVA outcomes for by phenotype for each species. Asterisk denotes a significant effect in the model. Full tables can be found in supplementary material.
Figure 1: Means for development time (A,B), female mass and lipid content (C,D), and male mass and lipid content (E,F) with *D. melanogaster* on the left and *D. simulans* on the right. Within each set of eight panels the four panels on the left show the response to media separated by photoperiod and the four panels on the right show photoperiod separated by media. Isofemale lines are colored by origin and are: red for the south, green for the mid-Atlantic and blue for the north.
Figure 2: The reaction norms for development time (A,B) female mass (C,D) and male mass (E,F) separated by species *D. melanogaster* on the left and *D. simulans* on the right. Within each set of eight panels the four panels on the left show the response to media separated by photoperiod and the four panels on the right show photoperiod separated by media. Isofemale lines are colored by origin and are: red for the south, green for the mid-Atlantic and blue for the north. The lines from the regions that displayed the greatest response in the meta-analysis are most prominent to better visualize the range of responses within a population.
Figure 3: The strength of response for development time separated by time spent as a larva (A) and time spent in the puparium (B). *D. melanogaster* is represented by open symbols while *D. simulans* is represented by closed symbols. The strength of response to media is represented by the triangles and circles represent the response to photoperiod. There is a clinal pattern for the ability of larva to respond. Although the time spent in the puparium is plastic there is no difference between populations.
Figure 4: The strength of response for development time separated by mass (A;B) and lipid content (C;D) as well as sex with males on the left and females on the right. *D. melanogaster* is represented by open symbols while *D. simulans* is represented by closed symbols. The strength of response to media is represented by the triangles and circles represent the response to photoperiod. Although both mass and lipid content are plastic with difference between populations there is no clinal signature. The exception is mass in *D. simulans*, which has a weak cline in female mass and a stronger cline in male mass.
CHAPTER 3

POLYMORPHISM IN THE COUCH POTATO GENE MEDIATES THE PLASTICITY OF LIFE HISTORY TRAITS IN DROSOPHILA MELANOGASTER

Abstract

The evolution of phenotypic plasticity is a possible adaptive outcome from environmental heterogeneity in natural populations. Despite the widespread occurrence of phenotypic plasticity, the genetic architecture and specific molecular variants that underlie plasticity are largely unknown. Genes that are pleiotropic and determine life history syndromes represent candidates for the mediation of adaptive plasticity. In Drosophila melanogaster a single nucleotide polymorphism at the gene couch potato (cpo) affects the expression of reproductive dormancy and a series of correlated, fitness-associated traits. This polymorphism also varies predictably in frequency in natural populations over multiple environmental gradients. In this study, the focal cpo variants were fixed in replicate genetic backgrounds to examine the effects of this polymorphism on aspects of life history and behavior. Larval diet was manipulated to evaluate patterns of plasticity for the focal polymorphism and to assess whether patterns of plasticity were consistent across a series of integrative traits. The results demonstrate that both cpo genotypes respond plastically to rearing environment in qualitatively distinct ways, suggesting that this polymorphism contributes to the genetic variance for plasticity in natural
populations. Furthermore, the low-diapause genotype was associated with a stronger response to rearing environment more often than its high-diapause counterpart.

Introduction:

The environment mediates how genetic variation is translated into the phenotypic variation upon which selection may act. This environmentally sensitive production of alternative phenotypes is referred to as phenotypic plasticity (Bradshaw 1965; Stearns 1989; West-Eberhard 2003). Elucidating the mechanisms that result in the expression of phenotypic plasticity is essential to a comprehensive understating of evolution and adaptation in response to environmental heterogeneity. This synthesis is especially needed for life history traits, which are characteristically complex, polygenic and can be particularly sensitive to environmental perturbations such as changes in temperature, nutrition, competition and predation regime (Roff 2002). However, it is often unclear if such phenotypic changes are adaptive, non-adaptive or neutral. Reaction norms in stressful and novel environments may reflect a physiological breakdown of the ability to reach the optimal phenotype rather then adaptive plasticity (Ghalambor et al. 2007). Furthermore, rarely are genes identified that underlie these adaptively plastic responses (Scheiner and Holt 2012). As such, it is necessary to dissect the genetic basis of variable trait response, as well as variance across traits, to better understand the variety of genes that underlie the plastic response and thus the evolution of plasticity.

The relevant response in the evaluation of plasticity is not the value of a trait but rather the difference in the trait produced by the same genotype across multiple
environments. Different genotypes may have diverse phenotypic responses across environments, which leads to an interaction between the genotype and environment (G X E). It is the lack of parallelism in the response, the G X E interaction, which provides the variation in response necessary for the evolution of plasticity via natural selection (Via and Lande 1985). When the mean trait value is plotted as a function of environment, those genotypes with a steep slope have generally been considered more plastic whereas those with a shallower slope are considered less responsive and less plastic (Pigliucci 2005). However, natural populations experience multiple environments, either simultaneously or in succession over developmental time. Thus, G X E interactions reflect the complexity of how multiple environmental pressures may interact non-linearly (Dorn et al. 2000).

Ultimately, the ability to produce an adaptive plastic response has a genetic basis. Model organisms such as Drosophila and Arabidopsis have been successfully used to identify and test candidate genes for plasticity. Both systems have the advantage of extensive genetic and genomic resources as well as natural populations that cover broad geographical and environmental ranges that allow for the examination of the evolution and maintenance of plasticity. Quantitative trait loci (QTL) mapping has been used to identify several loci that mediate trait value and that are sensitive to environmental change in Drosophila melanogaster (Leips and MacKay 2000; Bergland et al. 2008), Arabidopsis lyrata (Leinonen et al. 2013) and Arabidopsis thaliana (Stratton 1998). Candidates directly identified in mapping studies, as well as candidate genes inferred from other methods, have been used to demonstrate that expression of specific genes can affect plasticity. The natural polyphenism in flowering time in Arabidopsis has been
attributed to the *FRIGIDA* (*FRI*) (Johanson et al. 2000) and *FLOWERING LOCOUS C* (*FLC*) (Michaels and Amasino 2001) loci as well as to the epistatic interaction of *FRI* with *FLC* (Caicedo et al. 2004). Loss of function in these genes negates the ability to respond to environmental cues for vernalization (Le Corre et al. 2002; Micaels et al. 2003). Similarly, work with the insulin-like growth factor signaling pathway in *D. melanogaster* has demonstrated that decreased expression through the use of RNAi to decrease expression of the gene *forkhead transcription factor* (*foxo*) reduces the ability to modulate organ size in response to nutritional cues (Tang et al. 2011). However, to understand the evolution of plasticity it is essential to connect the identification and analysis of candidate genes with natural variation segregating at these loci in natural populations (Mitchell-Olds and Schmitt 2006).

Reproductive diapause is one example of life history trait polyphenism in *D. melanogaster* with candidate genes known to affect the ability to enter diapause in response to the environment (reviewed in Flatt et al. 2013). Diapause in *Drosophila melanogaster* is a reproductive quiescence elucidated in response to short days and cold temperatures (Saunders et al. 1989). The propensity to diapause varies predictably with latitude (Schmidt et al. 2005a) as well as season (Schmidt and Conde 2006), with diapause expression occurring more frequently in the spring and in higher-latitude populations. The genetic variance for diapause expression is correlated with other life history traits such as mass, stress resistance and longevity (Schmidt et al. 2005b; Schmidt and Paaby 2008). This indicates life history trade-offs associated with the ability to diapause, such that flies from high latitudes invest more in somatic maintenance whereas flies from low latitudes invest more in reproductive effort (Paaby and Schmidt 2009). The
molecular basis for the variation in the propensity to enter diapause is not fully known, but there are several genes that contribute to the propensity to enter diapause (Williams et al. 2006; Tauber et al. 2007). The *couch potato* (*cpo*) gene exhibits a robust association with the propensity to diapause in North American populations, and variation in diapause expression was further mapped to two single nucleotide polymorphisms (SNPs) in the *cpo* gene, including the adenosine/thymine polymorphism at position 48034 (Schmidt et al. 2008). These alleles (48304A and 48304T) vary in frequency predictably with both latitude (Schmidt et al. 2008; Fabian et al. 2012; Cogni et al. 2013; Bergland et al. 2014) and season (Cogni et al. 2013) in North America but do not exhibit a cline in Australia (Lee et al. 2011). The 48034A allele, which was associated with an increased diapause incidence, is predictably at higher frequencies at higher latitudes and in the spring season.

The *cpo* gene is a putative RNA binding protein that has been linked to both the endocrine system (Harvie et al. 1998) and the peripheral nervous system (Bellen et al. 1992), further suggesting it is involved in environmental sensing and the subsequent phenotypic response.

The *Drosophila* diapause system can be used to investigate the nature of the molecular basis of life history plasticity: it is possible to evaluate whether patterns of plasticity for alleles at a known gene that regulates one aspect of plasticity, such as diapause expression, are consistent across other traits associated with organismal fitness. The 48034A allele of the *cpo* gene is associated with an increased propensity to enter diapause. Thus, if genes for plasticity behave like a switch we would expect the more responsive 48034A allele to be highly plastic with respect to many life history traits. Alternatively, if genes for plasticity exhibit complex dynamics allowing for tradeoffs,
non additive interactions across traits, or distinct generic networks, we would expect the 48034A allele to be responsive for only some phenotypes: likely those associated with stress tolerance and diapause expression, such as body size and lipid storage (Schmidt et al. 2008). Under this hypothesis the alternative allele, 48034T, may be more plastic with respect to phenotypes associated with reproductive behaviors such as development time, fecundity and resource acquisition. Distinguishing between these two hypotheses will give insight into the nature of plasticity genes and the underlying molecular variation that determine the plastic response: either they behave as an upstream switch for plasticity, or these genes feed into the complex tradeoffs observed in life history syndromes.

Here, we examine how the above-mentioned SNP in the couch potato gene can affect life history traits as well as the nature of the plastic response to rearing environment across various traits. To produce a phenotypic response, we used iso-caloric food derived from two food sources, strawberries and apples, that are both in the family Rosaceae and are utilized by D. melanogaster in temperate habitats. Resource availability limitation has been shown to produce a more canalized response across genotypes (Bergland et al. 2008; Auld et al. 2010). To address the effects of resource availability, the nutritional content was manipulated by changing the amount of available protein. To evaluate the difference in plasticity between the focal genotypes, we measured the response to changes in rearing media for five different phenotypes. Three of the phenotypes are integrative responses to larval environment: development time, body size and lipid content. Development time is one of the most important aspects of life history with far-reaching fitness consequences (e.g., Kingsolver and Huey 2008). Body size and lipid content are two measures of downstream metabolic outcomes from food acquisition.
and allocation during the larval stages and metamorphosis in the puparium. Both body size and development time have been shown to affect fitness in *D. melanogaster* and to respond plastically with pervasive G X E interactions in response to temperature (James et al. 1997) and density (Santos et al. 1994). The other two phenotypes investigated were aspects of behavior: locomotory behavior in a choice arena with distinct food-borne olfactory cues, and oviposition preference based on medium. Food choice is driven by context-dependent olfaction cues (Root et al. 2011). Oviposition choice has been shown to be context-dependent and to have fitness consequences for offspring (Schwartz et al. 2012). Here, we examine the effects of the focal, molecular polymorphism at *cpo* on patterns of plasticity for these life history traits. Of particular interest is how functional variation at *cpo* modulates the extent of the plastic response for individual traits as well as the binary expression across traits, thus addressing whether plasticity is trait-specific or represents an integrated response.

**Materials and Methods:**

Fly stocks and culture

*Drosophila melanogaster* females were collected from two orchards, Davis Peach Farm (DPF, Wading River, NY 40.96°N; -72.81°W) and Rocky Ridge Orchards (RRO, Bowdoin, ME 44.03°N; -69.95°W) to establish isofemale lines. Third chromosomes were extracted and placed into a common genetic background of *w;6326* using marker-assisted introgression (Schmidt et al. 2005a). These chromosomes were subsequently sequenced
for a portion of the coding region of the *cpo* gene and assigned as *cpo* alleles by a focal amino acid polymorphism at residue 462 (*cpo462Lys* and *cpo462Ile*) that was previously found to be associated with life history traits (Schmidt et al. 2008). This amino acid residue is specific to the *cpo* RH transcript, identified by *in situ* hybridizations and cDNA sequencing (Bellen et al. 1992); subsequent analysis by the modENCODE project does not appear to support the existence of this *cpo*RH transcript (Graveley et al. 2011). The removal of *cpo*RH as an alternatively spliced transcript reassigns the focal polymorphism as an intronic SNP 38bp outside the major coding exon (discussed in Cogni et al. 2013). Due to the ambiguous nature of this polymorphism, it is labeled here as 48034 (A/T), according to the position relative to the start codon according to the Drosophila genome release v5. From each of the independent populations, DPF and RRO, 15-background replaced, extracted lines homozygous for one of the focal *cpo* alleles were used to initiate a population cage. Thus, in each cage the X and 2\(^{nd}\) chromosomes were isogenic; the 3\(^{rd}\) chromosome was fixed and homozygous for either 48034A (A/A genotype) or 48034T (T/T genotype) at this nucleotide position, and the remainder of the 3\(^{rd}\) chromosomal background was randomized by recombination. The population cages were allowed to recombine freely for ten generations at room temperature on cornmeal-molasses food with 2% yeast by volume. The different source populations, DPF and RRO, act as biological replication for the observed effect of the allele on the phenotype. Thus we were able to focus on the effect of the focal polymorphism and are able to attribute the mean phenotypic differences between the A/A genotype and T/T genotype populations to the effects of that allele on the phenotype.
Life history trait measurements

Four different food types were used to elicit a phenotypic response: 1) apple, 2) strawberry, both made with the standard amount of yeast (2% yeast by volume), as well as 3) reduced yeast apple and 4) reduced yeast strawberry food, made with half the amount of yeast (1% yeast by volume) to yield a 2 by 2 design. Both types of media contained 1000 ml deionized water, 28.0 g agar, 67.7 g nutritional yeast and 4.97 g methylparaben dissolved in 99.4 mL 95% ethanol. Strawberry and apple have different calories per g with apples being more calorically dense; to correct for this the food was standardized to 680 kilocalories of fruit per 1000 ml water. To make the fruit mixtures iso-caloric, 2122.8 g of strawberries or 1306.6 g of cored apple were homogenized in a blender and deionized water was added to bring the total volume of fruit add to 2215.6 ml. The agar solution was heated to a rapid boil then the fruit mixture and yeast were added. This was then cooked for 60 min at a low boil, cooled to 55°C, methylparaben was added and then poured. This recipe yields foods that are similar in sugar and yeast content but with different fruit bases. The low yeast treatment, containing 33.9 g of nutritional yeast, is a lower quality food meant to generate another environmental parameter to elicit a response. Experimental flies were reared at 25°C under a light regime of 12L:12D in Percival I36VL incubators. Development time, food odor choice, oviposition choice, mass and lipid content were measured in 14 independent, experimental replicates for each of the 16 experimental units (2 populations by 2 genotypes by 2 food sources by 2 levels of yeast). The phenotypes were measured for all replicates and treatment combination in each of three successive generations, yielding a
total of 672 groups of measurements for each phenotype (16 experimental treatment combinations X 14 replicates X 3 generations).

Phenotypic assays were initiated by placing grape juice-agar plates with yeast paste (active yeast and water) in the population cages overnight to collect a cohort of eggs. To ensure uniform density eggs were individually transferred to a vial containing experimental food medium at a low density of 12 eggs per vial. Both the time to pupation and the time to eclosion were measured, yielding two estimates for development time. Larval stage was tracked daily until pupation; after the formation of pupae, the individual pupae were numbered and tracked until eclosion. Pupation and eclosion were assessed at 4-hour intervals until the last viable pupa eclosed. Once eclosed, adults remained in their rearing vial at 25°C and 12L:12D in mixed sex groups until age 1-5 days. The mixed-sex groups of adult flies were subsequently moved to empty holding vials. The adults were held for a period of 2 hours before being released into a choice arena. The arena consisted of a cubic cage where each side is 30.5 cm with four funnel traps, one in each corner. Two of the traps contained standard apple food and two traps contained standard strawberry food: the position of the traps was randomized. After a period of 24h, the numbers of males and females in each trap were counted and flies were sorted by sex under light anaesthetic. Males were then frozen and stored at -20°C, whereas females were sorted into seven replicates per treatment block for the oviposition assays described below.

To measure oviposition choice, females from each treatment block were held in an empty vial for 2 hours before being released into a small cubic chamber where each side was 12 cm. These chambers contained two 35mm plates of standard strawberry food.
and two plates of standard apple food in randomized locations within each cage. Females were able to sample both food types and choose where to lay eggs for a 24h period, after which oviposition plates were then removed and eggs counted. Grape-juice agar plates were then placed in the chambers over a 24h period to collect eggs for the next generation, after which females were collected and stored at -20°C for mass and lipid measurements described below.

Males taken from the food odor choice assay and females removed from the oviposition assay were stored at -20°C until they were weighed. Samples of either 5 males or 4 females were dried at 60°C for a period of 56±3 hours to obtain the initial dry-weight measurement using a Sartorius R160P balance and weighed to the nearest 1x10^{-5} g. Total body lipid content was measured using ether extraction (Robinson et al. 2000). The samples were then left for 24 hours in 0.5 ml of diethyl ether to extract lipids. Extracted flies were then dried for 24 hours and reweighed. Total body lipid content was calculated as the difference between the initial dry-weight and the weight following the ether extraction.

Statistical analysis

All statistical analyses were run using JMP v.9.0.0 (SAS Institute, Cary, NC). Not every egg survived to eclosion; both total and stage-specific survivorship were statistically equivalent across food types within yeast treatments and between genotypes for RRO and DPF population sources. Egg-to-eclosion survivorship was lower on reduced yeast food, but there was no significant difference between the source
populations, media, or genotypes; thus, survivorship was not included in the subsequent analyses. For development time, we modeled both the time spent as a larva and the time in the puparium. Development was assessed using a three way ANOVA food type x genotype x yeast level with generation as a random variable. Mass and lipid content were log transformed prior to analysis; this yielded results that were qualitatively identical to analysis using the residuals resulting from the regression of body size and lipid content (not shown). Both mass and lipid content were assessed using a three way ANOVA with generation as a random variable. Oviposition and food odor choice were assessed using logistic regression to generate an effects likelihood ratios test to quantify preferences. As RRO and DPF populations exhibited qualitatively identical behavioral responses to the experimental treatments, the data were pooled for the final analysis of both food odor choice and oviposition. In all of the general linear model analyses, generation was included as a covariate. We were not interested in modeling the interaction between generation and other predictors since in this design generations function simply as another level of replication.

Results:

Development

Total development time was partitioned into two components: the time spent as a larva and the time spent in the puparium (Figure 1). For development time in the puparium, only main effects of medium, yeast content, and genotype were significant along with the interaction between yeast content and medium (Table 1). Flies reared on
strawberry food developed faster than those reared on apple cultures. Interestingly, flies reared on the low-yeast diet spent less time in the puparium, which compensated for an extended larval phase. Larvae with the T/T genotype consistently spent more time in the puparium than the A/A genotype regardless of food media or yeast content (Figure 1). Furthermore, under reduced protein the pupal stage was shortened possibly to compensate for the extended time dedicated to larval development. While the cpo genotypes exhibited parallel responses to the different environments for time spent in the puparium, they demonstrated significantly different responses with respect to larval development time.

The genotype-by-yeast and the three-way interaction, genotype-by-yeast-by-culture medium interaction, were both observed for the time spent as larvae (Table 1). The time spent as a larva drives patterns of variation for total development time, for which there are significant genotype-by-environment interactions (Figure 1). When larvae were reared on strawberry media, for both control and reduced yeast content, the cpo genotypes exhibited qualitatively identical development time. The rapid development observed on strawberry media may preclude expression of developmental differences between genotypes. In contrast, cultures on apple medium demonstrated an extended development time and the cpo genotypes were significantly distinct on both control and reduced yeast content food. Furthermore, the two cpo genotypes also exhibited a differential response to yeast content when reared on the apple medium: T/T genotypes were moderately affected, showing a difference of 4h (148h±0.66 on control, 152h±0.69 on low; p<0.001), whereas A/A genotypes demonstrated a stronger response with a difference of 20h (140h±0.55 on control, 160h±0.69 on low; p<0.001). All of these
factors contributed to the significant genotype by yeast by culture medium interaction. Overall, the T/T genotype was more responsive to changes in medium whereas the A/A genotype was more responsive to changes in yeast content (Figure 1).

Body size and lipid content

Body size (dry mass) and lipid content (total body lipid content) represent two measures of downstream metabolic outcomes resulting from food acquisition and allocation during development. Generally, cultures reared on strawberry food increased mass but exhibited no consistent response in lipid content. As expected, males were significantly smaller and were characterized by reduced lipid content. Females also demonstrated a greater degree of plasticity for both traits, and therefore the sexes were analyzed separately.

Female mass was affected by culture medium and yeast content, with no significant effects for genotype or any of the interaction terms (Table 2). While the two cpo genotypes exhibited similar mass in three of the four experimental treatment combinations, female T/T genotypes were larger than A/A when cultured on apple medium with normal yeast content (0.40 mg ±0.052 T/T, 0.38 mg ±0.048 A/A; p=0.0042; Figure 2). Similar to female mass, culture medium and yeast content had significant effects on male mass. In contrast to what was observed for females, there was a significant interaction between cpo genotype and medium as well as a significant three way interaction between cpo genotype, medium, and yeast content. When cultured on control yeast, T/T males were larger than A/A males on the apple medium, but this
pattern was reversed on strawberry medium. When cultured on low yeast content media, males of the two cpo genotypes were the same size on apple medium. On strawberry medium, however, A/A males were significantly larger than T/T males (0.28 mg ±0.052 A/A, 0.26 mg ±0.033 T/T; p=0.0084). Thus, the manipulation of larval diet resulted in the expression of plasticity for body size, as measured by dry mass, for both sexes. However, the observed patterns were also highly sex-specific. Across treatment combinations, females exhibited a greater range of phenotypic expression and degree of plasticity than did males. The responses of the two cpo genotypes were largely parallel across the dietary manipulations and demonstrated no difference in the ability to respond. However, the expression of plasticity for males was dependent on cpo genotype where the T/T genotype males demonstrated a more pronounced response to changes in culture medium.

Female lipid content exhibited significant interactions of genotype-by-yeast interaction, yeast-by-culture medium, and medium-by-yeast (Table 2). Females with the T/T genotype were responsive to changes in yeast availability regardless of rearing medium: when reared on low yeast diets, females of this genotype stored fewer lipids (Figure 2). This pattern is consistent for A/A females when reared on low yeast, strawberry media. However, on apple media there was no effect of yeast content. As suggested by the significant yeast-by-medium interaction, the availability of yeast had an impact on the response to changes in rearing medium. In the control yeast treatments, A/A females were responsive to changes in medium (0.040 mg ±0.003 on apple, 0.054 mg ±0.004 on strawberry; p=0.011) whereas the T/T females did not differ in their lipid storage (0.055 mg ±0.003 on apple, 0.063 mg ±0.003 on strawberry; p=0.14). In contrast A/A females reared on the low yeast treatment showed no response to medium (0.042 mg
±0.002 on apple, 0.045 mg ±0.003 on strawberry; p=0.38) whereas the T/T females demonstrated differential lipid content between media (0.042 mg ±0.003 on apple, 0.035 mg ±0.002 on strawberry; p=0.044). Thus, the differential responses of the two focal genotypes to the experimental treatments suggest that while both genotypes show plasticity for female lipid storage, the T/T genotype exhibited a greater range of response.

Male lipid content was unaffected by genotype or any other predictor. The yeast-by-culture media interaction was the only significant predictor of male lipid content in the full model (Table 2). However, planned comparisons did demonstrate a differential response between the *cpo* genotypes. The T/T genotype was responsive to changes in yeast content but only in the context of strawberry media (0.041 mg ±0.003 on control, 0.026 mg ±0.002 on low; p<0.0001). Similarly, the T/T genotype was responsive to changes in media but only in the context of reduced yeast availability (0.039 mg ±0.003 on apple, 0.026 mg ±0.002 on low; p<0.0001). This pattern is observed due to the far reduced lipid content of the T/T males reared on strawberry food in low yeast conditions.

Thus, manipulation of larval diet resulted in the expression of plasticity for lipid storage with sex-specific patterns. Across treatment combinations, females again exhibited a greater range of phenotypic expression and degree of plasticity than did males. Plasticity for lipid content in males is solely dependent on the interaction of yeast, medium and genotype such that only the T/T genotype in low yeast conditions demonstrated a significant response to changes in larval rearing environment.

Overall, females were more plastic as they demonstrated a larger range of trait values in response to the experimental treatments. Patterns of plasticity for the two *cpo* genotypes demonstrate patterns that are trait-specific. Female responses in body size were
largely parallel between the genotypes. In contrast, patterns of lipid storage were highly context-dependent. For males, the expression of plasticity was dependent on \textit{cpo} genotype, with the T/T genotype showing a greater degree of plasticity. Males with the A/A genotype demonstrated no ability to respond to the dietary treatments. Taken together, these observations demonstrate that the focal polymorphism has pronounced effects on body size and lipid content across environments; however, the influence of the alleles and their degree of plasticity is highly sex-specific and context dependent.

Behavioral choices

In both behavioral arenas, three general outcomes were possible: flies could 1) actively choose the medium on which they were cultured, 2) demonstrate no preference, or 3) avoid the medium on which they were cultured. Across both assays, consistent and significant differences between the two \textit{cpo} genotypes were observed with respect to this behavioral choice. The T/T genotype exhibited a significant relative preference for the medium on which they were cultured, suggesting a response to odor-borne cues associated with previous exposure. In contrast, the A/A genotype did not exhibit a difference in relative preference associated with culture environment, showing the same preference whether the flies were cultured on apple or strawberry medium.

In the food odor choice assay, there was a significant effect of rearing medium and yeast as well as genotype-by-yeast and a genotype-by-medium interaction (Table 3). Flies with the A/A genotypes exhibited a general preference for strawberry food regardless of the food type on which they were reared as larvae. In stark contrast, T/T
genotypes demonstrated a significant relative preference for the food type on which they were reared (Figure 3). For both genotypes, the relative response was not affected by yeast content: T/T genotypes demonstrated a significant differential preference under both control and low yeast diets, whereas the A/A genotypes exhibited no relative preference. Thus, the results demonstrate a differential behavioral response for the two cpo genotypes, presumably based on odor cues associated with the different fruit-based media.

In the oviposition assay females were given a choice to lay eggs on either apple medium or strawberry medium. Females with the A/A genotype laid fewer eggs per female within the 24h period than those with the T/T genotype (1.27±0.19 A/A, 2.07±0.23 T/T; p=0.004). Across all four rearing media and for both genotypes, females preferred to oviposit on apple food plates. Similar to the food odor choice assay, females with the T/T genotype exhibited an increased preference for the food on which they were reared and females with the A/A genotype showed no preference (Figure 4). Interestingly, with lower protein availability the preference disappeared for the A/A genotype. This is another example of a gene-by-environment interaction where the food type the fly was reared on had an effect on oviposition choice; again, however, this behavioral response was specific to the T/T genotype (Table 4). Thus, in both behavioral assays flies with the T/T genotype demonstrated an increased preference for their rearing medium whereas the A/A genotype exhibited no preference. This demonstrates differential patterns of behavioral plasticity between the cpo genotypes in response to food borne cues.
Discussion:

In this study we examined how allelic variation at couch potato (cpo) modulated the extent of the plastic response for individual traits as well as the magnitude of the plastic response across traits, thus addressing whether plasticity is trait-specific or represents an integrated response. Our data demonstrate that a single polymorphism in the cpo gene derived from natural populations can mediate plasticity for several life history traits and behaviors. It should be noted that as the cages were started with 15 lines, these lines could have been fixed for other nucleotide variants at other positions on the third chromosome. However, the replication of the experimental design in two independent populations should minimize the likelihood of fixation of additional variants across both populations. As demonstrated in our results, each of the traits assayed responded plastically to changes in larval environment and no one genotype was consistently more responsive then the other in all contexts. However, looking broadly across the phenotypes assayed, we find that the genotype associated with low-diapause propensity (T/T) is overall more responsive than its high-diapause counterpart, the A/A genotype. The expression of diapause is elicited by exposure to low temperature and short photoperiods, and thus diapause expression itself represents a plastic response. As such, if genotype-specific patterns of plasticity are consistent across traits, the A/A genotype would be predicted to demonstrate greater plasticity for the variety of traits we measured here. This was not observed: the low diapause genotype demonstrated a greater degree of plasticity.

Although these data are limited to the four environments assayed, we can speculate about the environmental heterogeneity that characterizes the latitudinal gradient.
in eastern North America. Northern populations arguably experience the most
environmental heterogeneity due to the temperate winter: flies in these locales experience
a wider range of annual temperature fluctuation as well as more extreme photoperiods.
However, southern populations may experience a greater degree of heterogeneity in the
biotic environment, as drosophilid species diversity is relatively higher in southern
regions of North America and Europe, thus allowing for greater possibility of
interspecific competitive interactions (Markow and O’Grady 2006). Parasitoids have
also been shown to have a clinal distribution where parasitoids in the south of Europe are
more numerous and virulent (Kraaijeveld and Godfray 1999). Furthermore, southern
populations likely experience greater environmental heterogeneity in food availability
with a wider range of crop availability, fruit type, and potentially microbiome fluctuating
throughout the year. The greater responsiveness of the \( cpo \) \( T/T \) genotype, which
predominates in low latitude environments in North America, may be related to this
increased food substrate heterogeneity. Although the majority of patterns of phenotypic
plasticity in natural populations are ultimately polygenic with complex inheritance,
identifying the genetic changes that are in part responsible for these complex phenotypes
is necessary to understanding the evolution of plasticity. Our results suggest that the
mediation of plasticity for some aspects of life history in North American populations of
\( D. \) \( melanogaster \) is due, in part, to allelic variation at the \( cpo \) locus.
Life history plasticity

All five traits demonstrated a plastic response to the environmental treatments, and this was, in part, modulated by *cpo* genotype. The low-diapause T/T genotype exhibited a different trait response for larval development time, body size and lipid content, although the latter two were specific to males. Furthermore, only flies with the T/T genotype made a distinction between their natal food source and the novel food source in both behavioral assays. In contrast, the high-diapause A/A genotype exhibited a plastic response for larval development time as well as female lipid content. For the T/T genotype, these patterns of plasticity were determined primarily by differences between fruit based rearing media. For the A/A genotype, trait response varied principally in response to protein content. Thus, the *couch potato* (*cpo*) gene does not simply function as a switch that incites plasticity across a wide range of phenotypes; rather, each allele modulated plasticity in a context and trait specific manner.

In the behavioral assays, only the T/T genotype flies made a choice based on rearing media with an increased preference for the culture environment to which they were previously exposed, suggesting that they are responsive to their olfactory environment. Conversely, A/A individuals showed no preference between novel food source and a familiar food source. Elucidating the molecular basis of food finding behavior or oviposition is difficult due to the context dependent, complex, and often polygenic nature of behaviors. Many investigations into the genetic basis of complex behaviors involve whole gene knockouts via pharmaceutical or genetic manipulation to assess how a single gene contributes to normal behaviors (Sokolowski 2001; Bendesky...
and Bargmann 2011). Indeed, olfactory receptors have been shown to affect food finding (Root et al. 2011) as well as learning (Xia et al 2005). Perhaps surprisingly, olfactory receptors have also been shown to affect life history traits via insulin-insulin like growth factor signaling (IIS) possibly mediated by the genes *forkhead transcription factor (foxo)* (Libert et al. 2007) and the insulin receptor substrate, *chico* (Naganos et al. 2012). These findings complement our results, which show that a gene previously evaluated for its effects on life history is also shown to influence two aspects of behavior. However, research on olfactory receptors has primarily been conducted using knockouts, and segregating variation of functional significance at these genes remains to be identified.

Heritable natural variation for oviposition choice has also been demonstrated. However, identifying the relevant genes and networks involved in the behavioral response remains a work in progress (Miller et al. 2011). The sitter-rover system appears to act as a simple switch based on natural variation at a single locus, *foraging* (*for*) that sets larvae and adults on two different tracks for a suite of locomotory and foraging behaviors (Pereira and Sokolowski 1993). Polymorphism in *cpo*, similar to the sitter-rover polymorphism, is another natural polymorphism that has the ability to affect trap association and oviposition behaviors as a simple switch for a behavioral response. The *for* locus has also been shown to have pleiotropic effects on metabolic and gene expression plasticity (Kent et al. 2009).

Development time, mass and lipid content are downstream expressions of the metabolic process of acquisition and allocation of energy (Boggs 2009). In the context of plasticity, these loosely correlated traits represent independent opportunities to respond to the environment with the possibility of forming an integrated life history profile or
strategy. Life history theory suggests that a fundamental tradeoffs may exist between body size, development time and growth rate, such that organisms that develop more slowly mature at a larger size (Roff 2002). However, this trade-off is rarely observed within a species, especially when environmental conditions differ (Nylin et al. 1993; Nylin and Gotthard 1998; Scharf et al 2006; Jiménez-Cortés et al. 2011). For example, within *Drosophila* individuals with the sitter variant do not differ in larval body size compared to those with the rover variant despite different rates of food acquisition (Graf and Sokolowski 1989). Furthermore, selection on body size and plasticity for body size has been shown to be independent of development time, which suggests that the traits are not strongly correlated in *D. melanogaster* (Hillesheim and Stearns 1991). However, even within *D. melanogaster* these results are not consistent across studies. Other work has documented a positive relationship between body size and development under selection (Nunney 1996). A fast rate of larval development may still be associated with trade-offs: at older ages, both a higher rate of age specific mortality and lower fecundity both appear to be correlated with fast development time (Metcalf and Monaghan 2001).

In this study, flies that took longer to develop did not yield larger adults. On the contrary, those reared on strawberry food were both the fastest to develop and the largest in adult size, demonstrating a lack of a trade-off between development time and body size. Thus, we will examine plasticity for development time separately from plasticity in lipid storage and body size. Both *cpo* genotypes demonstrated plasticity for development time but were responsive to different environmental parameters. The T/T genotype was responsive to changes in media whereas the A/A genotype was sensitive to the manipulation of protein level. Both genotypes decreased their time in the puparium under
low protein conditions to perhaps compensate for a longer larval period. Development time in *Drosophila* is more labile in larvae and more canalized in pupae (Partridge and Fowler 1992; Mirth and Riddiford 2007). Development time is set by the time it takes for a larva to reach a critical size and is known to have a hormonal basis including the IIS and Target of Rapamycin (TOR) pathways (Edgar 2006). These pathways seem to mediate the response of larvae to their nutritional environment and affect lipid storage via the fat body (Mirth and Riddiford 2007). As in our results, differences in the magnitude of plasticity in total development time are based on changes to larval rather than pupal development time, highlighting the importance of environmental sensing during larval development.

Both genotypes demonstrate plasticity in body size and lipid content, but the ability to respond was sex dependent. For males, the T/T genotype showed greater responsiveness across environments for both body size and lipid content. Although body size in females was very responsive to the environmental treatments, they showed no differences in the magnitude of response between the genotypes. When females were assessed for lipid storage the A/A genotype was again more responsive to media and the T/T genotype was more responsive to changes in protein content. These differences between the sexes highlight the difference in their underlying biology as well as potentially distinct life history strategies. Other genes have been shown to affect plasticity for body size in both males and females. RNAi was used to knockdown the expression of *foxo*, a downstream transcription factor that regulates insulin signaling and is critical for the response to lower food availability. Only those individuals with the normal level of *foxo* transcription were able to decrease body and cell size in response to
limited resources (Tang et al. 2011). Similarly, QTL mapping used to identify genes involved in plastic response of thorax size to low-protein larval diet identified *dare*, a gene involved in ecdysone synthesis, as a candidate for regulating plasticity in body size (Bergland et al. 2008). Ecdysone had previously been implicated in controlling adult body size mediated through interaction with IIS (Freeman et al. 1999). Both of these findings do suggest the importance of the IIS in the regulation of plasticity for life history traits. Ultimately, in our experiments the *cpo* genotypes exhibited different responses to the rearing environment. However, the magnitude of plasticity was sex specific and varied across the traits examined.

Nature of plasticity

In the case of *FRIGIDA* (FRI), *FLOWERING LOCUS C* (*FLC*), in the *Arabidopsis* system, and *forkhead transcription factor* (*foxo*) in *Drosophila melanogaster*, lower transcription leads to a diminished ability to respond to environmental variations (Le Corre et al. 2002; Micaels et al. 2003; Tang et al. 2011). Higher expression across multiple tissues may give more opportunity for environmental sensing. The prediction is that the allele characterized by higher levels of transcription will exhibit a more pronounced response to environmental change. Previous work has shown that the *cpo* A/A genotype (high diapause) has lower expression than the T/T genotype (low diapause) (Schmidt et al. 2008; Behrman et al. unpublished). Thus, we might predict that the T/T genotype would be more responsive across environments. While the T/T genotype did exhibit a higher degree of plasticity for some traits, our
results demonstrate that both genotypes exhibit a plastic response for a subset of traits and environments. Thus, the allele-specific gene expression levels alone are an inadequate predictor of how genotype-specific patterns of plasticity.

Our data are consistent with the prediction that plasticity is complex and modular. Our results demonstrate that a single polymorphism, previously associated with one aspect of plasticity, is also associated with differential expression of five traits in response to variation in the larval environment. However, plasticity is shown to be trait, sex and environment-specific. While the specific functions of cpo are unknown, the observed effects on plasticity may be mediated through IIS, a widely characterized regulator of basic life histories including body size (Colombani et al. 2005), aging (Clancy et al. 2001; Min et al. 2008), biological rhythms (Zheng et al. 2007), forging behavior (Kent et al. 2009) and development (Slack et al. 2011; Paaby et al. 2014). Complementation studies have demonstrated an interaction between a component of the IIS, Dp110, and cpo for diapause as well as other correlated traits providing support for the ability of cpo to interact with insulin signaling. In screens for genes during up regulated during diapause, the function of the up regulated many genes are unknown suggesting that there maybe a more complex genetic architecture that connects cpo to IIS as well as other pathways and processes (Schmidt 2011). Ultimately understanding the flux through these gene networks could better elucidate the molecular mechanism by which cpo interacts with other genes to affect the organismal response to environmental differences. This system offers an excellent opportunity to examine how nucleotide polymorphism within a gene can impact fundamental aspects of the plastic response.
Table 1: Analysis of variance for hours spent in a developmental stage with generation as a random effect.

<table>
<thead>
<tr>
<th>Source</th>
<th>Time in larval stage</th>
<th></th>
<th></th>
<th>Time in the puparium</th>
<th></th>
<th></th>
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<td></td>
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<td>F</td>
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<td>DF</td>
<td>SS</td>
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Table 2: Analysis of variance for body size and lipid content with generation as a random effect.
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Table 3: Effects likelihood ratios test for food preference.
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<td>0.4226</td>
<td>0.5156</td>
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Table 4: Effects likelihood ratio test for oviposition preference.
Figure 1 Mean (± se) time to eclosion broken for control yeast (panel A) and low yeast (panel B) broken into its components, time spent as larva and time as pupa. In panel A, the closed circles represent A/A genotype open and the open circles represent T/T genotype. In panel B, the closed triangles represent A/A genotype and the open triangles represent T/T genotype. Note that the different responses in total development time between genotypes are mainly driven by the time spent as larva.
Figure 2: Mean (± se) mass for females (A) and males (C) as well as the mean (± se) lipid content for females (B) and males (D). Closed symbols represent A/A genotype while the open symbols represent T/T genotype. Circles represent flies reared on control yeast and triangles represent flies reared on low yeast.
Figure 3: The proportion (± se) of flies that chose the apple medium when reared on control yeast (A) and low yeast (B). Asterisks denote means that are significantly different in the within-model planned comparison. Filled bars indicate those reared on apple while the open bars indicate those reared on strawberry.
Figure 4: The proportion (± se) of eggs laid on apple medium when females were reared on control yeast (A) and low yeast (B). Asterisks denote means that are significantly different in the within-model planned comparison. Filled bars indicate those flies reared on apple while open bars indicate females reared on strawberry.
CHAPTER FOUR

CONCLUSIONS AND FUTURE DIRECTIONS FOR IDENTIFYING GENES THAT MEDIATE PHENOTYPIC PLASTICITY

The focus of this dissertation was to examine the phenomena of phenotypic plasticity in natural population of *Drosophila* to better understand the genetic basis of the plastic response. To address the genetic basis of plasticity I measured patterns of plasticity in natural populations. Natural populations maintain variation in the ability to respond plastically with the strength of response of development time showing a clinal pattern. This work also demonstrated that measures of effect size could be applied to plasticity data to better quantify the strength of response. The effect size analysis was better able to gauge the differences in strength of response while the ANOVA was useful for discussing trait values. Furthermore, the strength of response across life history traits was variable suggesting that genes mediating the plastic response do not operate as a simple switch. To confirm these results, *couch potato*, a candidate gene that varies along the cline and is known to control plasticity in diapause expression was selected to assess the ability of a polymorphism may influence the plastic response. This work again showed that plasticity is not a simple switch but a complex output likely the resulting from the interaction of many genetic pathways.

The results from the two chapters show the same pattern of response. The clinal patterns measured in chapter two found that development time showed a clear clinal signature such that flies of both species from the south showed a greater response to
changes in rearing media when compared to the northern population. Chapter 3 demonstrated that the T/T genotype, which is found more common in the south, is also more responsive to changes between apple and strawberry media compared to the A/A genotype, which is found more commonly in the north. This parallelism is also seen comparing the strength of response for body size and lipid content. In natural populations there is a difference in the mean trait value for these measurements however there is no clinal signature for either of these traits with the ability to respond being roughly equivalent. Similarly, in chapter three the flies respond to changes in food media however, the relative strength of response was sex specific and genotypes did not differ greatly. These similarities between the effect of the cpo genotype and patterns observed in natural populations suggest that cpo may play a large role in the mediation of plasticity for life history traits in natural populations.

FUTURE DIRECTIONS: IDENTIFYING MOLECULAR VARIANTS

Plasticity is a growing field with medical applications since the progression of many metabolic diseases, such as diabetes, high cholesterol, and obesity, are dependent on the environment and diet of the individual as well as their genes (Low, et.al, 2011; Reed et al. 2014). Most of the work done to identifying genes (Pigliucci and Schmidt, 1985; Tang et.al, 2011) and gene networks (Blackman et al. 2011) has been done using a candidate gene approach. Although powerful, the candidate gene approach is limited by
the knowledge of a trait, often gained from research in model organisms and the imagination of the investigator in determining which genes are likely to affect the trait of interest. Unbiased screens circumvent this by looking for genes without any preexisting assumptions about what genes are involved in the manifestation of the phenotype.

One of these unbiased techniques, called Quantitative Trait Loci (QTL) mapping has been used to identify areas of the genome that underlie plasticity for some life history traits including body size and ovarial number (Bergland et.al. 2008). As molecular techniques becoming less cost prohibitive, we are able to do exciting new work that integrates new genomic tools to address evolutionary theory with more creative methods. Association studies are an increasing utilized technique that has made great strides with advancements in computational techniques. An association study is a fine scale approach compared to QTL mapping because an association study is able to identify specific changes in the DNA that are responsible for the phenotype. This methods for identifying genes that are involved in plasticity to yield a more complete understanding of the relationship between genes and phenotypes which has a medical application in addition to significance to the study of evolution.

The novel approach of assigning a plasticity score via Hedge’s g may be used as the trait value input in association or QTL studies to identify the variants that are associated with strong or weak responses. Data from such an investigation may confirm the importance of genes studied using the candidate gene approach as well as identify new variants that affect the strength of the plastic response. Using the unbiased screen may indicate gene networks that underlie plasticity to address the prediction of the
importance of modularity in the evolution of plasticity. This can be accomplished in model organisms by using gene set analysis, a bioinformatics techniques to cluster genes into groups biased on their known associations. The identification of new genes will not only add to a limited list of genes that are implicated in plastic responses but it will also open up new avenues of exploration into the mechanisms. The ability of any new candidates would be confirmed via techniques similar to those utilized in chapter 3 of this work. This approach adds significantly to our knowledge of what types of genes affect the ability to be plastic. From these list we can start to look at similarities between the functions of the genes that mediate plastic responses shedding light on the “black box” that allows plasticity to manifest in individual organisms.
APPENDIX

Supplementary Table 1: Analysis of variance for hours spent in a developmental stage for *D. melanogaster* with line nested within origin, medium and photoperiod as a random effect.

<table>
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<th>Source</th>
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Supplementary Table 2: Analysis of variance for hours spent in a developmental stage for *D. simulans* with line nested within origin, medium and photoperiod as a random effect.
Supplementary Table 3: Analysis of variance for mass and lipid content separated by sex for *D. melanogaster* with line nested within origin, medium and photoperiod as a random effect.

<table>
<thead>
<tr>
<th>Females</th>
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<th>Lipid</th>
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Supplementary Table 4: Analysis of variance for mass and lipid content separated by sex for *D. simulans* with line nested within origin, medium and photoperiod as a random effect.
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