Temperature, Photoperiod, and Life History Traits in Drosophila subobscura

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Temperature, photoperiod, and life history traits in *Drosophila subobscura*

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Master of Science

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Temperature and photoperiod are generally reliable indicators of seasonality that have shaped the life histories of many temperate zone organisms. Anthropogenic climate change, however, may alter historical weather patterns and seasonal cues. Many studies have evaluated thermal effects, but fewer have also examined photoperiodic effects, on life history traits. Here I look at the interaction between these cues on insect development time, adult survival, and fitness. Because the degree of seasonal cue varies across latitude, I also examine developmental plasticity across latitudinal clines from Europe and North America of Drosophila subobscura using a two by two factorial design with long (16L:8D) and short days (8L:16D) at high (23°C) and low temperatures (15°C). I find that development time is dependent on both temperature and photoperiod but the low temperature/long day treatment revealed a dramatic and unexpected 4.5 day delay in eclosion. Fitness, estimated by the intrinsic rate of increase ($r$), showed a significant increase in response to temperature and a decrease in response to day length, and an interaction such that long-days reduced the effects of temperature. Additionally, cooler temperatures increased lifespan, and long-days reduced survivorship; temperature and day length interacted such that lifespan is relatively shorter in mismatched (long-cool, short-warm) conditions compared to matched conditions. These data highlight the importance of multiple abiotic factors in predicting species' responses to climate change.
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This Ph.D. is dedicated to my father Kenneth MacLean who reminded me to pursue my passion. While he passed away during the writing of this thesis, he was always the first to ask if I had “published anything yet”.
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Temperature, photoperiod, and life history traits in *Drosophila subobscura*:

Overview

Chapter one of my thesis will provide an overview of life history traits, local adaptation and plasticity. It will focus on plastic responses to temperature and photoperiod, and how these cues interact in temperate insects. I will then describe the study system, *Drosophila subobscura*, and its usefulness in addressing the question of how temperature and photoperiod interact with the plasticity of life history traits. Chapter two of my thesis will describe a series experiments conducted to test the potential interactive affects of temperature and photoperiod on key life history traits in *D. subobscura* from two continents and across three parallel latitudes. The research demonstrates a significantly negative interaction between temperature and photoperiod in seasonally mismatched environments.
Life History and Environmental Cues:

Organisms achieve broad geographic ranges through local adaptation and acclimation to local, seasonal climate patterns; namely though behaviorally, physiologically, or morphologically responses to environmental variables (Janzen, 1967; DeWitt & Scheiner, 2004; Angilletta, 2009). Seasonal climatic variability increases with latitude; as a result, organisms must migrate, go dormant, exhibit seasonal polyphenisms, or exhibit plasticity in fitness related traits to persist through inhospitable seasons. To cope with this phenomenon, plasticity increases with increasing latitude for many taxa (Murren et al., 2014). Typically, plasticity is measured as a function of a continuous environmental condition such as temperature or as a function of an environmental threshold such as critical photoperiod. Understanding which environmental variables determine to the plastic response in a given fitness component can help us better understand adaptation and acclimation responses.

I. Local Adaptation and Life History Tactics

Life history traits are the suite of traits that characterize how a population makes a living in a particular ecosystem, and they are shaped by the sum of evolutionary forces acting upon these traits within a population (Stearns, 1976). Life history theory tries to predict the mechanism for the highest fitness in the face of different environmental challenges (Stearns, 1976). A local adaptation can be described as an evolved trait that confers a fitness advantage on a population in its home environment, or habitat, regardless of potential fitness costs in others (Williams, 1966). Environmental
heterogeneity makes the local adaptation of life history traits essential in many habitats however evolutionary dynamics such as gene flow and genetic drift may limit the rate or the extent of adaptation.

The success of life history tactics can be measured at the individual or population level. Fitness is typically estimated using one of two metrics: $R_0$, which measures the net reproductive rate of the population or $r$ which measures the per capita rate of increase per unit time (Huey & Berrigan, 2001). For insects that are univoltine and produce one brood per year, measures of $R_0$ are more appropriate to approximate fitness. These data are determined by stage-specific life tables to calculate net population growth. For insects that are multivoltine and produce multiple broods per year, measures of $r$ are more appropriate to approximate fitness, especially if there are overlapping generations. In a closed population, where there is no immigration or emigration, $r$ measures the intrinsic rate of increase by incorporating age at first reproduction, life span, and development time to estimate population size (Smith, 2007).

The life history of many temperate insects is shaped by environmental variability in temperature cycles, seasonality, and plant phenology directly or indirectly affecting components of fitness; specifically the number of reproductive events in a season, the number of offspring in a brood, the age at maturity, the size of offspring, and the survival rate (reviewed in Angillette, 2009; Dell et al., 2011). Insects can adapt to local environmental conditions through evolved or plastic shifts in morphology, behavior, or physiology (Angillette, 2009). Many insects have adapted to different
conditions along latitudinal and elevation gradients through morphological differences in body size, coloration, and insulation (Watt, 1968; Berry & Willmer, 1986; Gillis & Smeigh, 1987; Ellers & Boggs, 2004). Additionally, many insects are able to acclimate to their seasonal environment through shifts in resource allocation, development, or performance (Tauber & Tauber, 1976; Lachenicht et al., 2010).

Environmental conditions determine the number of generations per year for most insects. In univoltine systems, the reproductive stage occurs in favorable (summer) conditions and then the adult or developing stage(s) enter dormancy, or diapause, when the conditions turn unfavorable. Diapause is typically determined by a critical photoperiod, temperature or combination thereof (Tauber & Tauber, 1972). Thus, the reproductive stage of univoltine insects need only be adapted to one set of seasonal environmental conditions. While multivoltine systems may diapause, they produce more than one generation per year and the reproductive stage may need to cope with different seasonal conditions in each generation. Local adaptation and acclimation to seasonal conditions are evidenced by population persistence, despite constraints on development time, reproductive output, and survival (Williams, 1966).

Population persistence may, in many cases, be the result of life history tradeoffs. Reproduction and survivorship are the two most energetically expensive processes and thus are opposed in a fundamental way. Trade-offs between reproduction and survival are found across many taxa from humans to small insects (Stearns, 1992). Life history theory assumes, for a population to persist in a given habitat, it has been pushed to a theoretical optimum between two opposing life history traits (Roff, 1992); however,
there are mechanistic and ecological constraints to adaptation (Kawecki & Stearns, 1993; DeWitt et al., 1998). Under optimal environmental conditions, energetic investment in reproduction is generally favored over maintenance. Alternatively, suboptimal environmental conditions, may favor investments in somatic structures for survival or dispersal at the cost of reproductive effort. For example, because *D. melanogaster* demonstrates a direct fitness cost to activation of the innate immune system, there is decreased immune function with increase age (Zerofsky et al., 2005). Life history tradeoffs can also be observed between age at reproductive maturity and development time (Stearns & Koella, 1986). There is strong selection to reproduce at an early age; however, forces governing development time including, seasonally available habitat, limit this pressure. The life history tactics employed by a population in response to these limiting habitat variables are particular to the set of environmental conditions encountered by that population (Kawecki & Stearns, 1993).

II. Plasticity

Phenotypic plasticity can facilitate population persistence in unfavorable environments (Pagel, 1991; Angilletta, 2006; Glanville, 2006; Crispo, 2007; Ghalambor, 2007). For multivoltine insects that experience varied conditions from generation to generation, plasticity can lead to the production of a phenotype or that is better suited for a particular environment. In order for a single population to meet different environmental challenges, adults may use reversible plasticity in physiology and behavior, or earlier life stages may use developmental plasticity to respond to
environmental cues (DeWitt et al., 1998; DeWitt & Scheiner, 2004; Auld et al., 2010). A reversibly plastic trait is a trait that is induced by one set of environmental conditions and lost under another. For example, thermal hardening in ectotherms can directionally shift the optimal metabolic rate towards the temperature to which they were hardened (Hoffmann, 2003; Sejerkilde et al., 2003). Behavioral plasticity, or the ability of an organism to alter behavior in response to a complex or changing environment, can also have dramatic fitness consequences. For instance, increasingly complex environmental landscapes may require greater plasticity in foraging behavior (Snell-Rood & Papaj, 2009). In contrast to reversible and behavioral plasticity, developmental plasticity is when adult phenotypes are determined by the developmental conditions. Specifically, insect body size may be partially determined by developmental conditions, namely temperature or resource availability (Stearns & Koella, 1986).

While plasticity may buffer against environmental variability, it may also be a necessary precursor to genetic assimilation for the adaptation of organisms (Pigliucci et al., 2006). Genetic assimilation is the process by which the plastically induced trait expression is canalized as part of a developmental pathway. A case of genetic assimilation was described in *D. melanogaster* when temperature was used to induce the production of a cross-veined phenotype (Waddington, 1953b). In selecting for the plasticity of the phenotype, he produced a line of flies that produced the, “abnormal phenotype in the absence of the abnormal environment” (Waddington, 1953b). Since this time, researchers have been interested in the mechanisms and implications of genetic assimilation in response to novel environments. While there is some evidence of genetic assimilation, most seminally Waddington and Schmalhausen (Waddington,
1942; Schmalhausen, 1949; Waddington, 1953b; Waddington, 1953a; Waddington, 1961), the underlying mechanism and evolutionary process has been the topic of debate since for decades (reviewed in Pigliucci et al., 2006). Genetic assimilation can be thought of as an outcome of evolution in response to a novel environment rather than a hindrance or a force that rivals natural selection (Pigliucci et al., 2006). Genetic assimilation may contribute to local adaptation to multivariate environments and can be detected by differences in population reaction norms.

A reaction norm is used to visualize phenotypic plasticity along an environmental gradient. The reaction norm plots the environmental variable on the x-axis and the phenotypic response on the y-axis to demonstrate the range of phenotypes across environments. The shape of the reaction norm be used to characterize the phenotypic differences across environments, or it could simply be the phenotype itself (Angilletta 2006). Thermal performance curves (TPCs) are special types of reaction norms where the trait is characterized by a function such as metabolic rate or run speed over a range of temperatures (Huey & Kingsolver, 1993; Angilletta et al., 2008). A change, or difference, in reaction norm can be characterized by a change in the slope, offset or shape, of the curve in response (Murren et al., 2014).

While, studies of life history plasticity have examined a number of environment types ranging from species interactions to elevation, temperature is far and away the most common environmental variable (more than 150 studies conducted with more than two environments (Murren et al., 2014). Temperature governs many of the processes that determine life history traits; metabolic rate, development time, size, and is a
continuous environmental variable allowing for measurements to be taken over more than two conditions.

III. Thermal Adaptation

Temperature can impose directional selection on plastically induced or genetically determined traits phenotypic responses to temperature are well documented (reviewed in Dell 2012 and Angilleta 2009). For most ectotherms, temperature has a direct effect on size, performance, tolerance, and reproduction (Angilleta, 2009). While local adaptation can produce different thermal sensitivities in these traits, the main effects are so common that “rules” have been described for them (Kingsolver, 2009).

Cooler temperatures increase size. The Temperature-Size Rule (TSR) states that the size of an organism is inversely related to environmental temperature (Ray, 1960) and is nearly universal across taxa (Atkinson, 1994). Various adaptive and non-adaptive hypotheses have been postulated to explain this rule. One adaptive hypothesis suggests that larger size may be tied to decreased competition as a result of thermally induced high juvenile mortality rates. While on non-adaptive hypothesis proposes that the larger size is simply a by-product of the cold slowing down the developmental process such that each cell is larger at the time of division (Gilbert, 2001; Angilletta et al., 2004). The response to temperature is not only occurring on the plastic level but also on the genetic level and this is encapsulated in Bergmann’s rule. Bergmann observed that size differences among endothermic species has an inverse relationship to latitude and is thought to be the result of thermal selection pressure to increase size and thus decrease
the surface area to volume ratio (and thus reducing the rate of heat loss) in cool, high latitude conditions (Bergmann, 1847). For small ectotherms, increased body size does little to insulate against external temperature, but it is possible that a larger size is adaptive as it generally increases fitness and survivorship (Peters, 1986). Regardless of this phenomenon is adaptive or the result of increased development time, lower temperatures and higher latitudes produce larger body sizes in insects.

Warmer temperatures increase speed. In addition to changing the body size of an organism, temperature directly affects performance. Most measures of thermal performance quantify a performance metric, such as run speed or metabolic rate; at two or more temperatures producing a thermal performance curve (see II. Plasticity). Most measures of insect performance across a range of temperatures show the same pattern. The metric increases with increasing temperature, it plateaus, and then it rapidly decreases. While warmer temperatures speed up cellular kinetics and thus metabolic rate, there is a limit to the benefit. High temperatures that exceed a physiological maximum, degrade proteins and induce cellular stress responses (Huey & Kingsolver, 1989).

Increased temperatures speed up development time for temperate insects, but they also decrease life span (Brown et al., 2004). Short-term exposure, or hardening, to extreme temperature can prepare insects for the seasonal environment they will encounter next. Thermal hardening can initiate a physiological shift of thermal performance curve (Paulsen, 1968; Hoffmann, 2003; Sejerkilde et al., 2003; Mitchell et al., 2011; Arias et al., 2012). Rates of locomotion, chill coma recovery time, even
starvation resistance have all been shown to shift as a function of extreme temperatures (as adults or larvae) and are adaptive (Bubliy & Loeschcke, 2005; Loeschcke & Hoffmann, 2007).

III. Photoperiod Adaptation

Photoperiod is a reliable indicator of season and directly affects aspects of life history. Photoperiod can entrain circadian rhythms (Kaiser & Cobb, 2008) and alter survivorship (Sheeba et al., 2000). In some Diptera it can also alter growth and metabolic rates, with limited variation across latitudinal clines (Lanciani et al., 1990; Niegula & Johansson, 2010). Critical photoperiod can induce diapause, arresting development or reproduction in insects, but can be used facultatively depending on other environmental variables. The majority of work done on photo cues in insects has looked at circadian clocks and seasonal adaptations.

Circadian rhythm is a biological phenomenon wherein processes exhibit daily patterns and are entrained by exposure to a light dark cycle. There is a large body of work pointing to the importance of circadian rhythms (reviewed in Wager-Smith & Kay, 2000). The disruption of circadian rhythm can have deleterious consequence and as a result, circadian rhythms have been studied extensively in Drosophila (reviewed in Wager-Smith & Kay, 2000). Holding populations of flies in constant darkness results in decreased life span compared to the populations that were entrained with a circadian rhythm (Kumar et al., 2005). The underlying genetic mechanisms of the circadian clock
and its effect on locomotion and activity has been well studied in *Drosophila* as a pleitropic trait (reviewed in Jordan et al., 2006).

In addition to circadian rhythms, natural populations encounter circannual changes in photoperiod. Higher latitudes experience more extreme changes in day length between seasons relative to lower latitudes. As a result, higher latitudes tend to have shorter growing seasons and increased abiotic constraints on growth and development. Researchers who looked at a northern and a southern population of the damselfly, *Lestes sponsa*, in a common garden and observed that longer photoperiod decreases growth rate in regardless of native latitude (SNiegula & Johansson, 2010). Additionally, research with *D. melanogaster*, demonstrated that longer photoperiod decreases metabolic rate across four experimental temperatures allowing for increased survivorship and fecundity (Lanciani et al., 1990). This result suggests thermal compensation because the longer photoperiod would be associated with warmer summer temperatures which should act to decrease survivorship suggesting that photoperiod may be working counteract temperature in a seasonal environment (reviewed in Clarke, 2003). Thermal compensation is the ability of an organism to maintain physiological function in spite of exposure to different thermal environments (reviewed in Clarke, 2003). Increasing day length signal the beginning of the growing season and increased temperatures, while decreasing day length signal the end of the growing season and decreased temperatures. For organisms who have not entered a dormant or diapause state, thermal compensation mediated by photoperiod may be key for in energy conservation in high temperature conditions and for locomotion in low temperature conditions (Lanciani et al., 1991; Lanciani et al., 1992).
Diapause is a state of hormonally induced dormancy manifested by arrested development in the larval form. It is physiologically mediated through persistently high levels of Juvenile Hormone (JH) in a species specific instar to arrest development, or through the reallocation of reproductive effort to maintenance in diapausing adults, to potentially increase survivorship throughout a less favorable season (Tauber & Tauber, 1976). Diapause is typically induced when day length decreases below a threshold called the called critical photoperiod. However, in common garden experiments, many species enter a state of diapause dependent upon both photoperiodic and thermal cues (Bradshaw, 2004). In many cases, these cues are inter-dependent. For example, in high altitude populations of *Drosophila ananassae* both thermal and photo cues are used to entrain eclosion rhythms, but which cue is used depends on the threshold temperature (Khare et al., 2002). Apart from this research, relatively little is known about the interaction between temperature and photoperiod.

IV. Interaction between temperature and photoperiod adaptations

Temperature and photoperiod both directly affect life history and local adaptation to environmental conditions. While there are many studies that pair temperature and photoperiod to evaluate seasonal effects on phenotype or life history there, relatively little work has been done describing the potential interaction between temperature and photoperiod (Barker & Herman, 1976; Kingsolver & Wiernasz, 1991; Nylin & Gotthard, 1998; De Block & Stoks, 2003; Bradshaw, 2004). Research suggests that high temperature and short days increase development time relative to high
temperature and long day environments in damsel flies (De Block & Stoks, 2003). This result was surprising given the work done on fruit flies suggesting that short days should increase metabolic rate, thereby decreasing development time (Lanciani et al., 1991). This unexpected result suggests that the interaction between temperature and photoperiod is complex and needs further study.

The interaction between temperature and photoperiod is of increasing interest to both ecological and evolutionary biologists because of anthropogenic climate change. In addition to increases in mean temperatures, many climate projections predict an increase in extreme weather events and novel weather patterns that could result in novel temperature and photoperiod combinations (Williams et al., 2007; IPCC, Climate Change 2007). They could also result from species shifting their ranges poleward to seek thermal refuge. Range shifting in response to warming has already been reported across taxa (Wilson et al., 2005; Crozier & Dwyer, 2006; Gorman et al., 2010; Chen et al., 2011). As a result, some species are now experiencing longer photoperiods than ever before. Many studies predicting responses of species to climate change have focused on how increased mean temperatures will affect life history traits (reviewed in Parmesan, 2006; Andrew et al., 2013). However, these studies fail to recognize that photoperiod may interact with these increasing, or atypical, temperatures despite the evidence that photoperiod and temperature interact to shape life histories (Lanciani et al., 1990; De Block & Stoks, 2003; Bradshaw, 2004).

V. Study Species: Drosophila subobscura
Many studies of drosophilids show that higher temperatures decrease development time, increase reproductive output and decrease life span (reviewed in Atkinson et al., 1996). Longer photoperiod can reduce metabolic rate, while shorter photoperiod can increase it (Lanciani et al., 1990). There is also variation in responses to these environmental cues within populations and across species (Krebs & Feder, 1997; Schmidt et al., 2005). In order to investigate local adaptation and differential acclimation responses, evaluating samples from populations of Drosophila sampled across latitudes would be ideal.

Drosophila subobscura is a Palearctic species with a native range from North Africa to Scandinavia (Prevosti, 1955). In the late 1970’s a small propagule from a Southern Mediterranean population was introduced in South America near Puerto Monte, Chile (Brncic et al., 1981). This founding population retained only 25% of the ancestral European allelic diversity, but nonetheless rapidly expanded and today occupy much of Chile (Pascual et al., 2007). Shortly thereafter, in the early 1980s, individuals from this newly established population in South America were introduced to the western coast of North America (Beckenbach & Prevosti, 1986b; Pascual et al., 2007) (See Figure 1). Drosophila subobscura are currently distributed across a latitudinal gradient on three continents; Europe, South America, and North America. (Ayala et al., 1989b).

Despite the broad distribution of D. subobscura there is a high degree of gene flow among the ancestral European populations in nuclear, but not mitochondrial DNA (Latorre et al., 1992). There is no evidence, however, of ongoing gene flow among the
three continents (Pascual et al., 2007). Despite rates of intracontinental gene flow, both the ancestral and derived populations of *D. subobscura* have evolved clinal patterns in inversion polymorphism and wing length. (Prevosti et al., 1988; Gilchrist et al., 2004). Thus, *D. subobscura* affords the opportunity to evaluate clinal patterns of acclamatory responses across latitude on multiple continents.

*Drosophila subobscura* are relatively cold tolerant flies and do not diapause when over-wintering as adults (Lankinen, 1993; Goto et al., 1999). This allows for measures of fitness over a broad range of temperature and photoperiodic conditions without inducing immobilization or dormancy. Additionally, *D. subobscura* have continuous, overlapping generations such that all life stages experience a range of seasonal environments. Adaptive plasticity in response to seasonal environmental cues should be relatively common for life history traits in *D. subobscura*. *Drosophila subobscura* are ideal for characterizing the effect of a temperature and photoperiod interaction because of their broad geographic distribution and ability to withstand a range of environmental conditions.
Figure and Table Legends:

Figure 1: Adapted from Rodriguez-Trelles et al 1998. The white areas highlight the distribution of Drosophila subobscura across Europe, South America, and North America.
Temperature, photoperiod, and life history traits in *Drosophila subobscura*

**Introduction**

Temperature is a driving force in life history evolution, particularly for ectotherms (Tauber & Tauber, 1982; Roff, 1992). Specifically, temperature has effects on size, development time, lifespan, and fitness in insects (reviewed in Janisch, 1932; Angilletta, 2009; Dell *et al.*, 2011). Although insects generally have a restricted range of body temperatures over which they can achieve high rates of growth, foraging and other aspects of fitness (Andrewartha & Birch, 1954; Magnuson *et al.*, 1979; Huey & Hertz, 1984), they are nonetheless dispersed over a broad geographic range. Insects can adapt to local climatic conditions through heritable or developmentally plastic changes in morphology, voltinism, or physiology (Angilletta, 2009) Many populations also display reversibly plastic traits like behavioral thermoregulation, diapause, and thermal hardening (Hoffmann, 2003; Kellermann *et al.*, 2009; Mitchell *et al.*, 2011). Both genetically and environmentally determined traits show predictable patterns with regard to climate along both latitudinal and elevation gradients (Watt, 1968; Berry & Willmer, 1986; Gillis & Smeigh, 1987; Schultz *et al.*, 1992; Ellers & Boggs, 2004).

Temperature varies with some regularity along latitudinal gradients but photoperiod is a more reliable indicator of season in mid-to high latitude localities. Longer days are associated with summer and relatively warmer temperatures, while shorter days are associated with winter and relatively cooler temperatures. As a result, many organisms, including insects and plants, use photoperiod as a predictor of seasonal changes that can induce behavioral or physiological acclimation (Paulsen,
Photoperiod often cues dormancy or diapause, allowing organisms to conserve their energy and increase stress resistance during less hospitable months (Tauber & Tauber, 1982; Hoffmann & Sgro, 2011; Williams et al., 2014). Both the duration and temperature during diapause can have direct consequences for life history (Bradshaw, 2004; Williams et al., 2014). As a result, most studies of photoperiodic effects on insects have focused on diapause (reviewed in Tauber & Tauber, 1982), with the exception of work in *Drosophila* showing that long day photoperiod can decrease metabolic rate and increase thermal optima (Lanciani, 1990; Lanciani et al., 1991; Lanciani & Anderson, 1993). These researchers posit that the effects of photoperiod are adaptive, even compensatory, as they dampen the direct effects of temperature (Lanciani et al., 1992; Clarke, 2003). Thermal compensation, or the ability of an organism to maintain physiological function in different thermal environments, appears to be induced by photoperiod and mediated through metabolic rates. In these studies, thermal compensation allows for the conservation of energy in high temperature environments and increased performance in low temperature environments (Lanciani, 1990; Lanciani et al., 1991; Lanciani & Anderson, 1993).

Less is known about the interaction between temperature and photoperiod on insect life history traits. Although increased temperatures tend to decrease development time, decrease survival, and increase population growth rate, longer photoperiod can act to suppress metabolic rates. A study using damsel flies suggests that individuals reared in high temperature and long days develop more slowly than those in high temperature and short day environments (De Block & Stoks, 2003). If photoperiod is being used as a
cue for compensatory modification of thermal sensitivity, then short days, might trigger a physiological decrease in development time, potentially through increased metabolic rate. Therefore, I hypothesize that at low temperatures, short days will decrease development time, decrease survival, and increase population growth rate relative to long days.

The broad seasonal and geographic range of many Drosophila species, coupled with well-developed experimental techniques and interesting ecological attributes makes members of this group compelling candidates to quantify any implications for life history traits. Here, I use Drosophila subobscura from the ancestral European and the colonizing North American populations (reviewed in Ayala et al., 1989a), sampled from three latitudes on two continents to quantify the effect of temperature, photoperiod, and their interaction on key life history traits. These populations exhibit clinal patterns in body size, wing length, and inversion polymorphism despite high rates of gene flow within continental populations (Huey et al., 2000; Gilchrist et al., 2001; Balanya, 2006; Pascual et al., 2007). The recent introduction of the North American population allows me to examine variation in plastic responses across latitudes and between continents. The theory of thermal compensation predicts and generally finds that high latitude conditions are associated with reduced development times in common garden experiments (Śniegula et al., 2012). Latitudinal variation is of particular interest as recent anthropogenic climate change is projected to increased environmental stochasticity and temperature variability (Williams et al., 2007; Coumou & Rahmstorf, 2012; IPCC, Climate Change 2007), while photoperiod maintains its celestial regularity.
How does photoperiod affect the responses of life history and fitness traits in the context of the effects of temperature? To address this question, I reared flies from laboratory populations under four temperature-photoperiod regimes. I exposed replicated samples from six mass laboratory populations of *Drosophila subobscurea* (three from each continent) to all combinations of temperature (High 23°C versus Low 15°C) and photoperiod (Long 16L:8D versus Short 16L:8D ) treatments. The long photoperiod day is like a day in early May in northern Washington State, whereas the short photoperiod day is like a mid December day at the same latitude. Thus, the factorial design created analogs of a warm summer day, a cool winter day, and the novel, mismatched conditions of a warm winter day, and a cool summer day. A control line was reared at standard laboratory conditions (18°C, 14 L:10 D). Eggs were reared to adulthood in each of these treatments and were monitored for length of development time. The adults were assayed for fitness (estimated by the intrinsic rate of increase, *r*) and survivorship. Temperature effects on drosophilids are well characterized: low temperatures are associated with longer developmental times, lower intrinsic rates of increase, and longer survivorship (Bateman, 1972; Hoffmann, 2003). However, many animals have the ability to adjust their physiology in anticipation of seasonal change. If photoperiodic effects on plasticity are interacting with temperature effects, then short days, which signal winter temperatures, might trigger seasonal thermal compensation thereby increasing metabolic rate and resulting in decreased development time, higher population growth rates and decreased survivorship. By quantifying the responses to different combinations of photoperiod and temperature, I will begin to tease apart the relative contributions of each variable in acclimation responses. The use of three
populations from two continents allows me to examine geographic variability in these traits.

Methods

Study System:

The ancestral populations of *D. subobscura* range from North Africa to Scandinavia, where they have been shaped by over 10,000 years of post-glacial environmental variability in their native habitat (Prevosti, 1955). In the late 1970’s *D. subobscura* colonized Puerto Monte, Chile (Brncic et al., 1981); genetic analysis suggests that the flies were Mediterranean in origin and suffered a severe bottleneck event (Pascual et al., 2007). Nonetheless, populations rapidly spread out from Puerto Monte, spanning over 10° of latitude by the mid 1980’s (Ayala et al., 1989b). *D. subobscura* were discovered on the western coast of North America in the early 1980’s (Beckenbach & Prevosti, 1986a; Pascual et al., 2007), with populations ranging from Central California up the Pacific Coast and into Southern Canada. These flies appear to be derived from the introduced South American populations (Pascual et al., 2007). Despite the large geographic distance and the evidence of morphological clines, there is a high degree of gene flow among clinal populations of *D. subobscura* within each continent (Latorre et al., 1992; Pascual et al., 2001; Zivanovic et al., 2007). *D. subobscura* overwinter as adults but have no known diapause regardless of latitude of origin and thus no critical photoperiod (Lankinen, 1993).

The laboratory populations were established from approximately 20 gravid females collected from six natural populations along two parallel latitudinal clines from
Europe (Aarhus, Denmark at 56.15°N, 10.22°E; Lille, France at 50.63°N 3.07°E; Valencia, Spain at 39.43°N, -0.37°E) and North America (Port Hardy, British Colombia at 50.70°N, -127.42°E; Bellingham, Washington at 48.74°N, -122.47°E; and Gilroy, California at 37.01°N, -121.58°E) in the summer of 2004. They were maintained in the laboratory as large populations \( N_e \gg 200 \) at 18°C and 14L:10D cycle in population cages (25 cm X 14 cm X 12 cm) containing 100 ml of yeast/cornmeal/molasses media. Eggs were collected from these population cages within 18 hours of being laid. For each population, 500 eggs were distributed equally into 10 vials containing 10 ml of media. Two vials per population were then placed in each of the four experimental combinations of temperature (High 23°C versus Low 15°C) and photoperiod (Long 16L:8D versus Short 16L:8D) as well as the control condition, and reared to adulthood. The flies were maintained in five Percival environmental chambers, each programmed for one of the five combinations of photoperiod and temperatures. Temperatures were monitored using Hobo data loggers within each chamber, with adjustments made to keep each chamber within ± 0.5°C of its target temperature throughout the day.

*Development Time:*

Development time was calculated from day of oviposition to the date and time of adult eclosion for each treatment. For each population, 500 eggs were distributed equally into 25 25x95mm vials with 10ml of media (20 eggs per vial). These vials were then distributed among the five photoperiod-temperature regimes and the control chamber. This was repeated over three days to ensure enough eggs and that the larvae
were kept at low density to minimize competition. After pupation was observed, vials were checked every 8 hours (08:00, 16:00, 24:00) and any freshly emerged adults were counted and sexed. Each vial was scored until no more adults emerged.

Survivorship:

To quantify survivorship, 30 flies (15 male and 15 females) from each population and larval treatment were sexed during light CO₂ anesthesia 48 hours after eclosion and placed in a survivorship cage and immediately returned to their rearing environment. A 25x95mm vial with 10ml of molasses-yeast agar medium with 0.15mg of active yeast was affixed to the side of the cage and replaced weekly. The flies were counted daily; dead individuals were removed and lost individuals were censored from the study. The study continued for 90 days and a censored survivorship model was used to account for the flies that remained alive or escaped in the course of the study.

Intrinsic Rate of Increase:

To quantify population growth rate, I employed the Model II serial transfer assay outlined in Muller and Ayala (1981). Flies from each of the six populations were reared at low density (20 eggs per 5mm vial) in each temperature and photoperiod combination (15°C, 8L:16D; 23°C, 8L:16D; 18°C 14L:10D; 15°C, 16L:8D; 23°C, 16L:8D). Adults were collected three to six days post-eclosion and sorted by sex into groups of 10, using light CO₂ anesthesia. The flies were then returned to their experimental treatment conditions and given three days to recover before being combined with the opposite sex, for a total of 20 flies, in a 50 mL bottle of cornmeal-
molasses agar with 23 mg of water diluted yeast paste. There were two vials per replicate and 3 replicates conducted from July 2008 through March 2009. Then, following the Model II serial transfer method (Mueller, 1981), all adults were removed, sexed, and counted once every seven days for eight weeks to obtain estimates the intrinsic rate of increase \((r)\).

For the Mueller assay, I first estimated lambda, the finite rate of increase or the rate at which a population size can change over one time step

\[
\lambda = \frac{N_{t+1}}{N_t}
\]  

(1)

where \(N\) is the population size, and \(t\) is time (or generation). Population growth rate across experimental weeks is estimated by the linear equation

\[
N_t = a_1 N_{t-1} + a_2 N_{t-2} + a_3 N_{t-3} + \ldots + a_i N_{t-i}
\]  

(2)

where \(a_i\) is the constant per capita output of an \(i\)-week old vial. As \(t\) gets larger, the per capita growth rate that is obtained is independent of the initial \(N\) and is estimated by the first positive eigenvalue of equation 2

I used a jackknife to estimate variance in \(\lambda\) between the replicates; I deleted the \(j\)th set of observations and calculated the leading eigenvalues (as above) yielding \(\lambda_j\). Then I calculated \(m\) pseudovalues as

\[
s_j = m\lambda - (m-1) \lambda_j
\]  

(4)

where \(j = 1, 2, \ldots, m\).
The jackknifed estimate of the largest eigenvalue is the mean of the pseudovalues giving an estimate of $\hat{\kappa}$ for all replicates. (Mueller, 1981).

$$\hat{\kappa} = (1/m) \sum_j s_j$$  \hspace{1cm} (5)

For overlapping generations over time, the intrinsic rate of increase ($r$) is estimated as:

$$r = N^{-1} \frac{dN}{dt} = \ln \hat{\kappa}$$  \hspace{1cm} (6)

**Statistical Approach**

All statistical analyses were performed in *R version 2.9.1* (Grambsch, 2014). For development time, I used a linear mixed effect model with the nlme package (Pinheiro, 2014) to assess the main and interactive effects of temperature, photoperiod, latitude and continent as predictor variables. This allowed me to estimate the main effects of temperature and photoperiod while testing for variability between continents and across latitudes. The predicted decrease in development time at higher temperatures would be indicated by a negative temperature term in the linear model. The predicted decrease in development time at short photoperiod would be indicated by significant positive effect of photoperiod in the linear model. If there is an interactive effect of temperature and photoperiod, I would expect to see an increase in development time on long days that is greater at colder temperatures. If there is clinal variation among populations, I would expect to find evidence of thermal compensation that is stronger with increasing latitude. Thus, I would expect that increasing latitude would shorten development time, and would be indicated by a negative latitude term in the linear model. Finally, if there
is variation between continents, I would expect the continents to have different intercepts in the linear model.

I calculated and compared survival probabilities for each treatment using the “survreg” function in the “survival” library in R version 2.9.1 (Grambsch, 2014). Day of death was examined as a function of population, temperature, and photoperiod. I tested for a main effect of continent and did not find one so it was used as a random effect or “strata” in the analysis. The predicted decrease in survival at higher temperatures would be indicated by a negative temperature term in the linear model. The predicted decrease in survival at short photoperiod would be indicated by significant positive effect of photoperiod in the linear model. If there is an interactive effect of temperature and photoperiod, I would expect to see an increase in survival on longer days that is greater at colder temperatures. If there is clinal variation among populations, I would expect to find evidence of thermal compensatory evolution, with compensation increasing with latitude. Thus, I would expect that increasing latitude would increase survival, and would be indicated by a positive latitude term in the linear model. Finally, if there is variation between continents, I would expect the continents to have different intercepts in the linear model.

For intrinsic rate of increase, I used the estimated \( r \) as the vial level response variable. I estimated \( r \) for all lines based on an eight week period starting with 20 adults. This was repeated three times for each line in each treatment. I again used a linear mixed effects model with the nlme package to assess the main and interactive effects of temperature, photoperiod and latitude and continent as predictor variables (Pinheiro, 2014). The predicted increase in population at higher temperatures would be indicated
by a positive temperature term in the linear model. The predicted decrease in population growth rate at short photoperiod would be indicated by significant positive effect of photoperiod in the linear model. If there is an interactive effect of temperature and photoperiod, I would expect to see an increase in population growth rate on longer days that is greater at warmer temperatures. If there is clinal variation between populations, I would expect to find evidence of thermal compensation that is stronger with increasing latitude. Thus, I would expect that increasing latitude would increase growth rate, and would be indicated by a positive latitude term in the linear model. Finally, if there is variation between continents, I would expect the continents to have different intercepts in the linear model.

Results:

Development Time:

Development time in D. subobscura is longer, than that of many other well-studied Drosophila species. The laboratory stock populations held at 14:10 LD and 18C typically take 20 days from egg to eclosion. Even though males eclose first in many holometabolous insects, I found no significant effect of sex on development time in any of the populations (figure 3, Sex: $F_{(1,60)}=0.07, p =0.786$). Nor did I observe an effect of continent, or latitude on development time (figure 3, Continent: $F_{(1,60)}=0.35, p =0.55$, Latitude: $F_{(1,60)}=0.38, p =0.541$). As expected, lower temperature ($F_{(1,60)}=1279.09, p <<0.001$) as well as long photoperiod ($F_{(1,60)}=10.46, p =0.002$) increased development time. The mean delay caused by longer photoperiod was only 0.34 days at high temperature but 4.79 days at low temperature, demonstrating the predicted interactive
effect of temperature and photoperiod to delay development time ($F_{(1,60)}=9.16, p=0.003$) (Figure 1).

**Survivorship:**

While I found no significant effect of continent (deviance=1.30, df=1, $p=0.253$); I did observe a positive effect of latitude on survival probabilities such that populations from higher latitudes, survive longer on average (deviance=9.44, df=1,$p=0.002$). As expected, I observed that lower temperature increases survival probability (deviance=266.22, df=1, $p <<0.001$) and shorter days increase survival probability (deviance=47.80, df=1, $p <<0.001$) at both rearing temperatures. The interaction between temperature and photoperiod decreased survival in the high temperature and short day treatment (deviance=66.55, df=1, $p <<0.001$); however, contrary to my prediction, the low temperature and long day treatment showed lower survival relative to the low temperature and short day (Figure 2). I predicted that the short day would increase metabolic rate and that higher metabolic rates are associated with lower survival. What I saw was that the seasonally matched, low temperature short day and the seasonally matched, higher temperature long day showed increased survivorship relative to the seasonally mismatched treatments.

**Intrinsic Rate of Increase:**

My estimate of $r$ did not vary with latitude ($F_{(5,144)}=0.82, p=0.537$). As predicted, I observed a significant increase in population growth rate ($r$) with high temperatures ($F_{(1,144)}=38.61 p<0.0001$). However, population growth rate ($r$) decreased
under long days ($F_{(1,144)}=69.55, p<0.0001$). Moreover, I saw a negative interaction between temperature and photoperiod such that high temperatures and short days produced the highest growth rate, and cold temperatures and long days produced the lowest growth rate ($F_{(1,144)}=5.005, p = 0.0271$, Figure 3).

**Discussion:**

The main effect of temperature in this study was not surprising given what is known about temperature and life history in insects. Hotter temperatures decreased *D. subobscura* development times and elevated the intrinsic rate of increase, but also decreased adult survival time. In our experimental treatment, the larvae in high temperature developed 11 to 15 days faster than those in low temperature, depending on photoperiod, regardless of latitude or continent of origin. Our measure of intrinsic rate of increase integrates both development time and reproductive output over an eight week period; high temperatures are associated with higher overall fitness. Previous research on *D. melanogaster* found that warmer temperatures increase reproductive output and decrease lifespan (Nunney & Cheung, 1997), as was observed in the present study.

Photoperiod is an important indicator of seasonality in the temperate zone; however, the effects of photoperiod on non-diapausing insects are little understood (Lanciani, 1990; Sheeba *et al.*, 2001). Here I observe that longer photoperiod increased development time, shortened adult lifespan, and lowered the population growth rates; long days had a detrimental effect on all of my measured life history traits. One of the few prior studies of photoperiodic effects on traits other than diapause showed that for
*D. melanogaster*, photoperiod can affect metabolic rates. Specifically, short photoperiods of 8L:16D elevate metabolic rates whereas long photoperiods of 16L:8D can suppress metabolic rates, which suggests thermal compensation (Lanciani, 1990; Lanciani et al., 1992; Clarke, 2003). Thermal compensation is generally adaptive and enables organisms to maintain their physiological function in the face of challenging thermal environments.

However, when I mismatched seasonal photoperiods and temperature, the results were not entirely as expected. Longer photoperiod significantly increased larval development time over short photoperiod at both test temperatures. Larval mass is the result of the acquisition and assimilation of resources, lower metabolic rate would increase the amount of time at each instar, and delay overall development. I observed this developmental delay in both the development time experiment and in the intrinsic rate of increase experiment. Because long photoperiods suppress metabolic rates, it is as if the animals are consuming a poor quality diet: no matter how much they eat, they cannot metabolize it as efficiently as animals on shorter photoperiods. Diet quality manipulations in other insects, specifically *Manduca sexta*, demonstrate that lower quality diets significantly delay development (Davidowitz et al., 2004).

Contrary to what I expected, long days were detrimental to survivorship at both temperatures in this study. Metabolic theory suggest that a lower metabolic rate contributes to a longer lifespan (reviewed in Finch, 1990). If long days decrease metabolic rate and if decreased metabolic rate increases life span, the prediction would be that longer days increase lifespan. This is completely unsupported by my data. Light increases activity in adult *D. melanogaster* (Martin et al., 1999), so longer days may
increase the amount of active time and thus lead to increased resource use that is unlikely to be compensated by adult feeding behavior; this could reduce adult life span relative to the short day treatments. The negative effect on lifespan would be accentuated at high temperatures, which impose an additional kinetic energy cost. That cost could be reduced by compensation such that long days, which normally co-occur with higher temperatures, might reduce metabolic rate. In this experiment, that compensation was relatively ineffective; long days, even at low temperatures, reduced survival over short days.

Despite summer conditions generally promoting population growth rate increased on shorter photoperiod at higher temperature. Given that shorter photoperiod decreased development time, at both temperatures I predicted that the intrinsic rate of increase might increase with higher temperature and shorter photoperiod. However, the observed decrease in development time was only a matter of hours at the high temperatures (Figure 1). This suggests that even though light increases activity in adult _D. melanogaster_, the increased metabolic rate of the adults may have increased egg-laying and copulation behaviors regardless of the shorter lights-on time (Martin _et al._, 1999).

While I observed no continental difference in life history under these regimes, I did observe a clinal difference in survival, which is consistent with some degree of local adaptation in lifespan response to lower temperatures of the high latitude populations. Estimates of gene flow among the European populations indicate panmixia in nuclear DNA. In contrast, mitochondrial DNA has relatively low gene flow (Latorre _et al._, 1992), suggesting that males may disperse longer distances than females. While this
could account for the lack evidence of local adaptation between latitudinal clines in plasticity for development time and population growth, it is more likely just a lack of resolution in the methods used here. Moreover, I found no significant difference in life history responses to temperature and photoperiod cues between continents, suggesting that all of these populations may respond similarly to environmental cues.

This study is one of the first to explore the interaction between two of the most important environmental cues, photoperiod and temperature, in temperate zone clinal populations from two continents. Prior studies indicate that the high temperature treatments would probably shorten developmental times, increase population growth rates, and decrease survivorship (Bateman, 1972; Hoffmann, 2003), however little is known about the effects of photoperiod aside from some evidence of thermal compensation (Lanciani, 1990; Lanciani et al., 1992). Based on these studies, I expected short day photoperiod, which increases metabolic rates, to decrease development time, decrease survivorship, and increase population growth rates. In line with my prediction for development time, I observed that at 23°C, photoperiod has little effect, whereas at 15°C, the long days prolong development by three to five days. In contrast to my expectation that short days would increase survivorship, the interaction between temperature and photoperiod led to decreased survivorship for both of the mismatched conditions (short-warm, long-cool) relative to their matched counterparts. Perhaps the metabolic demands in mismatched conditions resulted in decreased survivorship, given Lanciani's (1990) finding that a shorter photoperiod causes adaptive changes to counter the slowing kinetic effect of low temperature on metabolic rate (Conover & Present, 1990; Somero, 2004; Yamahira et al., 2007). Thus local adaptation
in metabolic rate may account for the latitudinal differences observed in survival.

Finally, in contrast with my prediction that short days would lead to a decrease in population growth rate at high temperatures, the greatest population growth rate ($r$) observed in this study was on short day photoperiod at 23°C.

These data introduce a new dimension to studies of thermal physiology. The quantification of thermal performance has generally been measured as a function of temperature and then extrapolated to ecological scenarios. Life history measures, such as the ones in this study, have become increasingly important in biophysical models of performance. Failing to take into account the effect of photoperiod, or assuming that it is independent of temperature, could lead to dramatic under- or over-estimation of performance in ectotherms under novel climatic regimes. Climate change presents a whole new suit of challenges for organisms that rely on environmental cues (Bradshaw & Holzapfel, 2006; Parmesan, 2006). Novel temperature and photoperiod combinations could occur as a result of extreme weather events and atypical weather patterns, both of which are expected to increase by 2100 (Williams et al., 2007; IPCC, Climate Change 2007). Moreover, species may experience novel temperature-photoperiod conditions as they shift their ranges poleward to seek thermal refuge. Range shifting in response to warming has already been reported across taxa (Wilson et al., 2005; Crozier & Dwyer, 2006; Gorman et al., 2010; Chen et al., 2011). As a result, some species are now experiencing longer summer and shorter winter photoperiods than ever before. These data suggest that simply considering temperature in calculating life history responses in these novel environments may not be sufficient. Organisms exist in a multivariate
environment and modeling potential responses to climate change require integrating environmental information and not just looking at temperature to make predictions.
Figure and Table Legends:

Figure 2: Development times for females and males. Temperature denoted in color- red being high temperature and blue being low temperature. Open circles (dashed lines) represent North American populations and closed circles (solid lines) represent European populations. Control treatment is shown in black.

Figure 3:
Survivorship curves for the European (top row) and North American (bottom row) populations of Drosophila subobscura going from low (left) to high (right) latitude. The dashed lines represent short days and the solid lines represent long days. The blue lines represent low temperature and the red lines represent high temperature. The solid black line is the control. As this is a censored survivorship assay, the crosses represent an individual that was censored from the population (i.e. escaped).

Figure 4: Intrinsic rate of increase plotted as a function of photoperiod (denoted on the axis) and temperature (denoted in color- red being high temperature and blue being low temperature). Open circles (dashed lines) represent North American populations and closed circles (solid lines) represent European populations. Control treatment is shown in black.

Table 1:
Summary of statistical linear mixed effects model and results for the development time, survivorship, and intrinsic rate of increase experiments
Development Time (d)

Figure 2
Figure 3
Figure 4
<table>
<thead>
<tr>
<th>Statistical Model</th>
<th>Development Time</th>
<th>Survival</th>
<th>Intrinsic Rate of Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature</strong></td>
<td>$F_{(1, 60)}=1279.09, \ p \sim 0.001$</td>
<td>deviance=$266.22, \ p \sim 0.001$</td>
<td>$F_{(1, 144)}=38.61, \ p \sim 0.0001$</td>
</tr>
<tr>
<td><strong>Photoperiod</strong></td>
<td>$F_{(1, 59)}=10.46, \ p = 0.002$</td>
<td>deviance=$47.80, \ p \sim 0.001$</td>
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</tr>
<tr>
<td><strong>Latitude</strong></td>
<td>$F_{(1, 50)}=0.38, \ p = 0.541$</td>
<td>deviance=$9.44, \ p = 0.002$</td>
<td>$F_{(5, 144)}=0.82, \ p = 0.537$</td>
</tr>
<tr>
<td><strong>Temperature * Photoperiod</strong></td>
<td>$F_{(1, 50)}=9.16, \ p = 0.003$</td>
<td>deviance=$66.55, \ p \sim 0.001$</td>
<td>($F_{(1, 144)}=5.005, \ p = 0.0271$)</td>
</tr>
</tbody>
</table>
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IPCC Climate Change 2007. Synthesis report. Contribution of working groups i, ii and iii to the fourth assessment report of the intergovernmental panel on climate change IPCC, Geneva, Switzerland: 104


Citations- Chapter Two:


evolutionary thermal biology. *Physiological and Biochemical Zoology* 79: 000-000.


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Vita

Heidi Joan MacLean was born in Riverside, California, on October 22nd, 1982, the second daughter of Marsha and Kenneth MacLean. After graduating from Redlands East Valley High School in 2000, she entered the University of Redlands in Redlands, California. In May of 2004 she completed her Bachelor of Arts in Biology. During the following years, she was employed by Fairfield Residential as a Recruiter before returning to school. In August of 2007, she entered graduate school at the College of William and Mary.