RAPID EVOLUTION AND POPULATION DIVERGENCE IN RESPONSE TO ENVIRONMENTAL CHANGE IN *COLIAS* BUTTERLIES

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology in College of Arts and Sciences.

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ABSTRACT

Jessica Keppel Higgins: RAPID EVOLUTION AND POPULATION DIVERGENCE IN RESPONSE TO ENVIRONMENTAL CHANGE IN *COLIAS* BUTTERLIES
(Under the direction of Joel Kingsolver)

My dissertation focuses on how environmental change, specifically in temperature and host plants, can drive physiological and morphological differences. I took advantage of historical studies with the *Colias* system of butterflies to assess adaptation and plasticity in larval performance in response to climatic change and changing host plant abundance. I have found that changing temperatures have affected the adaptation of some larval traits but not others. Specifically, as temperature variability has increased in both California and Colorado populations of *Colias,* the larval feeding rate has shifted to correspond to the new environmental conditions. Next, I studied how two Colorado populations of *Colias eriphyle* cope with repeated exposures to sub-lethal high temperatures simulating multi-day heat waves. I found that the higher elevation population suffered less detrimental fitness effects than the lower elevation population in regards to both short term (heat shock gene expression) and long term (overall growth rate) fitness effects. Building on my interest of how temperature and temperature variation affects multiple life stages I studied the effects of temperature during the pupal life stage on survival, growth and the resultant adult wing morphology. Generally, high temperatures decreased pupal time and less melanic adult wings. Finally, I used the two populations of *C. eriphyle* to quantify thermal performance differences of fitness when

larvae consume different host plants at two temperatures. I found that cooler temperatures increased the difference in performance between populations consuming different host plants and that thermal performance differs between populations. My research shows that temperature can affect fitness across many life stages and organisms have responded to these changes in temperature over time by adaptation.

To my mother who always inspired me to never stop learning.

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CHAPTER 1: OVERVIEW

Populations evolve traits that frequently yield a fitness advantage in their local environment, resulting in local adaptation (Williams 1966). Local adaptation to environmental conditions generates and maintains diversity among populations and species (Levene 1953). Organisms can be locally adapted to both abiotic factors such as climate and to biotic conditions such as competition, host plants, prey and/or natural enemies.

Temperature affects virtually all biological processes and systems in ectothermic organisms. Populations of the same and closely related species are often adapted to the local climatic conditions that they experience, resulting in clines in many phenotypic and morphological traits along latitudinal and elevational gradients. Local adaptation can also be seen on small spatial scales along elevational gradients has many taxa for a variety of traits, including phenology (Hodkinson 2005), morphology (Roland 1978), body size, behavior (Dingle, Mousseau & Scott 1990), thermal performance, and thermal tolerance (Damme *et al.* 1989; Stevens 1992; Gaston & Chown 1999; Badyaev & Ghalambor 2001). A major determinant of these local climates is temperature. In addition to temperature, insects can also be adapted to specific host plants or families of host plants. Local adaptation of insects to host plants is driven by aspects of host plant quality including abundance, plant defenses, and plant nutrient levels (Fox, Waddell & Mousseau

1994; Jongsma & Bolter 1997; Egan & Ott 2007). Herbivore populations may utilize and adapt to novel host plants that are introduced into their range.

Humans are changing the environment in many ways that are having interesting and diverse effects on organisms. As anthropogenic climate change increases both mean temperature as well as variability in climate and temperature, these novel conditions will present adaptation challenges for organisms (Easterling et al. 2000; IPCC 2007). For holometabolic organisms the effects of climate change may have both short and long-term effects that affect each life stage differently (Kingsolver, Arthur Woods et al. 2011).

Local adaptation of thermal optima is common across latitude and elevations (Huey & Kingsolver 1993; Cunningham & Read 2003; Sun & Friedmann 2005), however, ectotherms that were once adapted to their limited temperature range may now be experiencing fitness consequences as global mean temperature is increasing. The increasing temperatures along with the fact that upper thermal limits of performance in terrestrial ectotherms do not vary with elevation or latitude (Addo-Bediako, Chown & Gaston 2000; Sunday, Bates & Dulvy 2011) makes performance at temperatures above an organism's current thermal optima and below their upper thermal limits particularly interesting to study.

Human agriculture also changes the diversity and availability of host plants. Recent studies have demonstrated that evolutionary responses to novel host plants can occur quite rapidly, both for invasive plants (Harvey *et al.* 2010) and agricultural crops (Hare 1990; Gray *et al.* 2009). The interaction of temperature with other biotic factors such as availability and preference of certain foods can also affect local adaptation. Several herbivorous insects have demonstrated that host plant and rearing temperature

can interact to positively or negatively influence larval growth and development (Pelini *et al.* 2009; Diamond & Kingsolver 2012; Clissold, Coggan & Simpson 2013).

For my dissertation I want to know how populations are adapting to environmental change specifically changes in climate, host plants, and the interaction both. Overall, temperature and host plant choice are extremely important factors in understanding and exploring fitness differences across different populations and by using the *Colias* butterfly system I am able to elucidate some of the ways that temperature, changes in temperature, the availability of host plants, and how temperature and host plants interact can influence population fitness.

Colias butterflies have served as a model system for studying thermal adaptation for over 50 years (AE 1958; Hoffmann 1978). *Colias* adult butterflies and their thermal adaptation specifically regarding wing morphology is well characterized (Watt 1968; Kingsolver & Watt 1983; Kingsolver 1983; Ellers & Boggs 2002). However, little is known about *Colias* larvae and their local adaptation to climate (Sherman & Watt 1973). Additionally, *Colias* are generalists that feed on many plants in the *Fabaceace* family, however the distribution of genera is varied across their range. There is historical evidence suggesting that there is rapid local adaptation to host plant and local temperature (Sherman & Watt 1973; Tabashnik 1983) making the *Colias* system an attractive one to use to examine how thermal adaptation has changed due to climate change.

Colias (sulphur) butterflies range from lowland to alpine habitats across North America. *Colias eurytheme* is commonly known as the orange or alfalfa sulphur and is ubiquitious across North America below 2000m. *Colias eriphyle* occurs in open habitats

in the western US, and in western Colorado it is found at elevations of 1,400-2,900m. The larvae for both species feed on plants in the *Fabaceace* family, particularly *M. sativa* (alfalfa), *Vicia* (vetch) *spp., and Trifolium* (clover) *spp*. They have five larval instars and undergo a facultative diapause during the 3rd instar depending on local climate conditions. In my dissertation work I used these two species of *Colias* from four populations across the United States.

By using these species of *Colias* and the historical data available on local adaptation to temperature and host plants in larvae and plasticity in adults I am able to study changes local adaptation and plasticity over time. To do so in my dissertation I ask four major questions:

1. **Given the rate of climate change that has already occurred in the past 40 years, how has thermal local adaptation of *Colias* larval performance changed?**
2. **What are the long and short-term fitness effects of repeated exposure to sub-lethal high temperatures in *Colias eriphyle* larvae?**
3. **What are the effects of pupal temperature on pupal development and adult wing morphology in *Colias eriphyle?***
4. **What are the fitness effects of variable temperatures and the shift from a native host plant to an introduced host plant in Rocky Mountain *Colias eriphyle?***

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CHAPTER 2: GEOGRAPHIC DIFFERENCES AND MICROEVOLUTIONARY CHANGES IN THERMAL SENSITIVITY OF BUTTERFLY (*COLIAS)* LARVAE IN RESPONSE TO CLIMATE

Introduction

Populations are often adapted to the local climatic conditions that they experience, resulting in clines in many phenotypic traits along latitudinal and elevational gradients. Local adaptation over small spatial scales along elevational gradients has been documented in many taxa for a variety of phenotypic traits, including phenology (Hodkinson 2005), morphology (Roland 1978), body size, behavior (Dingle, Mousseau, and Scott 1990), thermal performance, and thermal tolerance (Stevens 1992, Badyaev and Ghalambor et al 2001, Damme et al. 1989, Gaston and Chown 1999).

Adaptation to climate is of increasing importance given the recent changes to regional and global climates, which are predicted to continue in the coming century (Easterling et al 2000, IPCC 2007). California, Colorado and other western states have shown a significant increase in the number of warm days and nights (where the maximum/minimum temperature is above the 90^{th} percentile recorded from 1961-1990) since 1950 (Booth, Byrne, & Johnson 2012). The ecological consequences of recent climate change have been abundantly documented for many regions and taxa and include changes in seasonal timing, life history traits due to plasticity, geographic distribution and abundance, and extinction risks (Parmesan and Yohe 2003, Walther et al. 2002). In many cases, climate change is causing mismatches between local adaptation to past climates

and new climate conditions. A natural question is whether evolutionary responses to recent climate change can reduce this mismatch. Recent studies have documented evolutionary changes in response to climate change in body size or phenology in birds (Charmantier et al. 2008), mammals (Reale et al. 2003), mosquitoes (Bradshaw and Holzapfel 2001), alpine plants (Anderson et al 2012), and herbivorous insects (van Asch et al. 2013). Some contend that evolution in response to seasonal cues rather than thermal adaptation will be most important for evolutionary responses to climate change (Bradshaw and Holzapfel 2007, Karell et al. 2011). To date, evidence for evolutionary responses in thermal physiology to recent climate change has been limited (Stillman 2003, Huey, Patridge and Fowler 1991). Whether this is because such evolutionary changes are infrequent or unimportant or because there is a lack of appropriate historical data on physiological traits remains to be determined.

Colias butterflies have served as a model system for studying thermal adaptation for over 50 years (Ae 1958, Hoffman 1978). These butterflies range from lowland to alpine habitats across North America. Previous work, however, has largely focused on adult traits. In the Rocky Mountains of Colorado adult butterflies of *C. eriphyle* and closely related species demonstrate morphological adaptation to temperature in wing melanism and thorax fur thickness (Watt 1968, Kingsolver 1983a, Kingsolver 1983b). Little is known about *Colias* larvae and if they also display local adaptation to climate (Sherman and Watt 1973). Rates of larval feeding, growth, and development are essential to success, and are strongly temperature-dependent in most insects (Stamp and Casey 1993). The primary function of the larval life stage is to assimilate nutrients, and larvae do this by near-constant feeding. Sherman and Watt (1973) measured short-term rates of

larval feeding in two *Colias* species: *C. eurytheme* from the Sacramento Valley in California (19 m) and *C. eriphyle* from the Montrose Valley in Colorado (1,633 m). *Colias eriphyle* had lower optimal temperatures for feeding (23-25°C) than *C. eurytheme* (29-31°C), suggesting local adaptation to the differing thermal conditions in these areas. By re-measuring larval feeding in these populations today, we can examine whether the thermal sensitivity of larval feeding has shifted in response to climate change in these areas during the past 40 years.

Here, we examine two *C. eurytheme* and two *C. eriphyle* populations differing in elevation and physiological adaptation to temperature by quantifying thermal performance curves (TPCs) of short-term feeding rate. Our goal is to see how well physiological traits are adapted to local climate, specifically temperature. We predict that the TPCs for each population cover the range of temperatures experienced during the growing season. In addition, we compare our data on TPCs for two of these populations, *C. eurytheme* from the Sacramento Valley, CA and *C. eriphyle* from the Montrose Valley, CO, with historical data (Sherman and Watt 1973) collected in 1971. We expect changes in the TPC for feeding rate to reflect the changes in climate over the past 40 years. As warm temperatures have increased in these regions, we predict that the larvae will be able to continue feeding at these new higher temperatures. This would be indicated in the TPC by a rightward shift to a new higher optimum temperature (T_{opt}) while retaining the same overall shape. Changes in TPC due to increased temperatures over the past 40 years could demonstrate how rapid evolution for a thermally important trait could potentially ameliorate the effects of climate change.

Materials and Methods

Study System

Colias eurytheme and *C. eriphyle* are sister species and occasionally hybridize in populations where they co-occur (Wheat and Watt 2008). The larvae for both species have five larval instars and *C. eriphyle* undergo a facultative diapause during the 3rd instar whereas *C. eurytheme* overwinter as quiescent larvae. The larvae for both species feed on plants in the *Fabaceace* family, particularly *M. sativa* (alfalfa), *Vicia* (vetch) *spp., and Trifolium* (clover) *spp*.). *Colias eurytheme* is commonly known as the alfalfa butterfly and is ubiquitous across North America below 2000m. *Colias eriphyle* occurs in open habitats in the western US, and in western Colorado it is found at elevations of 1,400-2,900m.

We collected *Colias* from four sites for these studies. To allow historical comparisons with Sherman and Watt (1973), we sampled *C. eriphyle* females from alfalfa (*Medicago sativa*) fields located in the Montrose Valley, CO (N38.62, W108.02, 1,633 m); and *C. eurytheme* females from alfalfa fields in the Sacramento Valley, CA (N38.44, W121.86, 19 m). To expand the geographical and climate range of our study, we also considered an additional site for each species: *C. eriphyle* from a county park with meadows including vetch (*Vicia)* and clover (*Trifolium)* near Gunnison, CO (N38.56, W106.94, 2,347 m); and *C. eurytheme* from an organic farm in Chapel Hill, NC (N35.87, W79.20, 148 m). In North Carolina *C. eurytheme* hybridizes with sympatric *C. philodice*. Hybrids often show mixed wing patterning and various levels of orange pigment on the ventral forewing (Gerould 1943, Hovanitz 1949). Based on emergence dates and wing morphology we classify our specimens from North Carolina as *C.*

eurytheme, however without DNA evidence to support this it is possible that we could have *C. philodice* and *C. eurytheme* hybrids.

These four study sites have different growing seasons, which account for variation in larval development, adult flight time, and the number of generations per year (voltinism). In the Sacramento Valley, CA the growing season (defined as the time for larval development and adult flight time) is essentially continuous resulting in 8-9 generations of *C. eurytheme* per year. In Chapel Hill, NC, the season starts in starts in March and ends in November resulting in 3-5 generations of *C. eurytheme* per year. In the Montrose Valley, CO the growing season can start as early as April and continue through October resulting in 3-5 generations of *C. eriphyle* per year. The shortest season is in Gunnison, CO, starting in June and continuing through September resulting in two generations of *C. eriphyle* per year.

Measurements of feeding rates

Adult female butterflies were collected from each site and shipped overnight to our laboratory at the University of North Carolina at Chapel Hill (butterflies from Chapel Hill, NC were driven to the laboratory). The female butterflies were kept in cages at greenhouse conditions (~26°C) under natural light. Females were fed 10% honey water solution by moistened sponge changed daily, and were allowed to oviposit on potted *Vicia villosa* in the greenhouse. Eggs were removed each day and placed in environmental chambers (Percival 36VL, Geneva Scientific, WI, USA) maintained at 25°C on a 14L:10D photoperiod where larvae were given leaves of *V. villosa ad libitum*. Upon entering the 5^{th} instar, larvae were starved for three hours and weighed. The larvae were then exposed to one of 5-10 different experimental temperatures between 15°C and

35°C and allowed to acclimate for 15 minutes before cut *V. villosa* leaves were added. Once the *V. villosa* was added the larvae were allowed to feed for 30 minutes. To ensure experimental temperatures above the optimal temperature for feeding were included, some populations were measured at several additional temperatures between 38°C and 43°C.

After the trial, larvae were removed from their temperature treatments, weighed again and placed back into the 25°C chamber and given *V. villosa ad libitum* until the next day. Each larva was tested at least twice at a different temperature for each feeding trial with occasional larvae going through the experiment a third time. Experimental temperature treatments were chosen and ordered randomly for each larva to avoid lumping potentially stressful temperatures at a certain larval age. Our sample sizes were as follows: for Sacramento Valley, CA N=90 larvae in 296 feeding trials, for Chapel Hill, NC N=92 larvae in 235 trials, Montrose Valley, CO N=168 larvae in 401 trials, and Gunnison, CO N=134 in 334 trials.

Our methods of assessing short-term feeding rate differed in two ways from the previous Sherman and Watt (1973) study. First, Sherman and Watt quantified feeding rate (mm^2/s) by measuring the time required to consume a fixed surface area of leaf of *V. villosa*. As a result, the length of the feeding trial varied with temperature. Because of the difficulties of accurately and repeatedly measuring surface areas for the highly divided *Vicia* leaves and leaflets, we instead measured feeding rate as larval mass gained, over a fixed (30 min) feeding trial. The larvae were starved prior to each trial, and there was no frass production during the 30 minute trial, thus mass gained directly reflects consumption. Second, Sherman and Watt (1973) measured body temperature by

inserting thermistor probes into individual caterpillars and heating them under spot lamps. Caterpillars were measured multiple times, but the number of caterpillars included was not reported. Our current experiment was conducted in controlled environmental chambers at different constant temperatures, which were maintained throughout a given feeding trial. By measuring each individual 2-4 times over a range of temperatures, we can estimate the magnitude of individual variation within populations. These methodological differences will lead to quantitative differences in feeding rates (including maximal rates of feeding) in the two studies, but should not affect the position (e.g. optimal temperature) or shape (e.g. thermal breadth) of the TPCs (see below, and Discussion).

Field temperature data

We obtained daily minimum and maximum air temperatures for the appropriate growing season of each population (Sacramento Valley, CA: January-December, Chapel Hill, NC: March-November, Montrose Valley, CO: April-October, Gunnison, CO: May-September) from 1961-1971 (Sacramento Valley, CA and Montrose, CO only) and 2001-2011 (all sites) from weather stations within 25 km of our field sites (National Climate Data Center, Global Historical Climatology Network-Daily). We created a sawtooth linear curve between each daily minimum and maximum and evaluated the curve at each 0.1 of a Julian day to estimate the temperature density for each population during the growing season.

Analysis

All data were analyzed using the R (15.1) statistical package. Feeding rate was defined as $F = \frac{ln(Final\ Mass/Initial\ Mass)}{Time\ Spent\ Feeding}$. This represents the proportional rate of mass

gain of a larva. Feeding rates were analyzed with linear mixed effects models using the nlme package. The model used for our feeding rate analysis was $F \sim T+T^2+T^3+P+T:P+T^2:P+T^3:P$, where F=feeding rate, T=temperature, P=population, and T^2 and T^3 signify temperature squared and cubed respectively. Note that the population term indicates differences among populations in overall rate of feeding, and interaction terms indicate differences between populations in thermal sensitivity of feeding rate. Because individual larvae were measured multiple (2-4) times, family as well as individual within family was included as random effects in the model however these effects did not significantly affect the model outcome (family, $\sigma = 0.012$, individual within family, $\sigma = 0.015$). For the historical comparison the model used was $F \sim T+P+Y+T^2+T^3+T:P+T:Y+T^2:P+T^2:Y+T^3:P+T^3:Y$ which included the Y=year term.

To characterize the differences in feeding rates among populations, we estimated key parameters describing the mean thermal performance curve (TPC) for each population. We used the TPC model proposed by Frazier, Huey, and Berrigan (2006), which is the product of a Gaussian function and a Gompertz function:

$$F(T) = F_{max}\, e^{-e[\rho(T-To)-6]-\sigma(T-To)^{\wedge}2}$$

Where F(T) is the feeding rate at experimental temperature T, F_{max} is the maximum feeding rate, T_o is the optimal temperature, and ρ and σ determine the thermal sensitivity of feeding at temperatures above and below T_o, respectively. The parameters were estimated using the nls function in R for each population. Using these values, we also computed thermal breadth B_{80} as the temperature range for which the feeding rate is 80% of the maximal rate F_{max} (Hertz, Huey, and Stevenson 1993; Bauwens et al. 1995).

Results

Differences in thermal performance curves among current populations

Thermal performance curves (TPCs) for feeding rate differed substantially and significantly among populations (Figure 2.1, Table 2.1). There were significant 1st and 2nd order effects of temperature on feeding rate, reflecting the unimodal shape of the mean TPC for each population. Populations differed significantly in their overall rates of feeding across temperatures, as indicated by the significant population effect. Importantly, there were also significant interactions between population and temperature, indicating differences among populations in the shapes of their TPCs (Fig. 2.1).

These differences in TPCs can be characterized in terms of the key parameters (Frazier, Huey, and Berrigan, 2006) that describe thermal performance curves (Table 2.2). Comparing all four of the populations shows that the maximum feeding rate (F_{max}) was lower for the Sacramento Valley (low elevation) population of *C. eurytheme* than for the other three populations. When just looking at the within species comparisons, optimal temperature (T_{opt}) was greater for Gunnison (high elevation) than the Montrose Valley (low elevation) population of *C. eriphyle*, and lowest for the Sacramento Valley population of *C. eurytheme* (see Table 2 for note about Chapel Hill). Conversely, thermal breadth (B_{80}) was greatest for the Sacramento Valley (low elevation) population of *C. eurytheme*, and smallest for the Montrose Valley (low elevation) population of *C. eriphyle*.

Patterns of field temperatures

Larvae from all populations except Montrose Valley fed at temperatures in the lab that exceed the climatic temperatures (T_{air}) they would normally experience in the field during their growing seasons (Fig. 2.2). The *C. eurytheme* populations experience longer

growing seasons (365 days and 275 days for Sacramento Valley and Chapel Hill populations, respectively) than the *C. eriphyle* populations (214 days and 122 days for Montrose Valley and Gunnison populations, respectively). The broad TPC of the Sacramento *C. eurytheme* population enables feeding at a substantial rate during both hot summer conditions and during the cooler conditions in spring and fall, however, our feeding rates were never directly measured in the field (Fig. 2.2, left panels). Note that the T_{air} distributions in both the *C. eurytheme* sites, Sacramento Valley and Chapel Hill, have a single strong mode, especially in summer, reflecting the higher humidity and reduced diurnal temperature fluctuations at these sites. In contrast T_{air} distributions in the two *C. eriphyle* populations were strongly bimodal (or multimodal), especially during the growing season, reflecting the greater diurnal temperature variation at these drier Colorado sites. The TPCs of *C. eriphyle* suggest that these populations fed substantially only at temperatures in the higher mode: they were capable of feeding at air temperatures during the day, but not at night. This effect was particularly noticeable for the higher elevation (Gunnison) *C. eriphyle* population. Interestingly, *C. eurytheme* at Sacramento Valley and *C. eriphyle* at Montrose Valley consistently experienced T_{air} near or above their optimal temperatures (Fig 2.2); the other two populations rarely experienced T_{air} close to their optima (but see Discussion).

Historical comparison

For two populations-- *C. eurytheme* from Sacramento Valley and *C. eriphyle* from Montrose Valley-- we compared the short-term rates of larval feeding previously reported by Sherman and Watt (1973) with our current results. Thermal performance curves for feeding rate differed significantly between time periods (years) for each population,

although for Montrose Valley, CO the overall shape of the TPC remained constant as reflected by similarities in ρ and σ despite the curve shifting in response to increasing temperatures (Fig. 2.3, Table 2.3). The significant 1^{st} and 2^{nd} order temperature terms show differences in unimodal curvature. The interactions between the 2^{nd} order temperature terms and year indicate significant differences in TPCs between the previous and current data (Table 2.3). Both populations have increased their capacity to feed at higher temperatures during the past 40 years (Fig. 2.3). In addition, during the past 40 years, T_{opt} increased by ~ 3°C in *C. eriphyle* at Montrose Valley, while for *C. eurytheme* it did not change, whereas, thermal breadth increased substantially in *C. eurytheme* at Sacramento Valley, with only a small increase at Montrose Valley (Table 2.2). The F_{max} results are not directly comparable from 1972 to 2012 because feeding rate was measured using different metrics in the two experiments. These results indicate that the positions and shapes of TPCs for larval feeding have changed substantially in these populations during the past four decades.

Air temperature data show that climate conditions have also changed during the past four decades at these sites (Fig. 2.3). While overall mean temperatures during the growing seasons show slight increase from the 1960s to the 2000s (18°C to 19.5°C in CA, and 13.5°C to 14.5°C in CO) and temperature variation has increased more dramatically, reflecting a change from unimodal to multimodal distributions at both sites. In addition, the frequency of higher temperatures has increased markedly at both sites. For example, the frequency of air temperatures above 28°C has increased from 8.8% to 18.2% at Sacramento Valley and 4.4% to 20% at Montrose Valley. As a result, climate

change has increased the frequency of exposure to high air temperatures by two- to four-fold at these sites (Fig. 2.3).

Discussion

Population divergence and climate differences

We evaluated larval local adaptation to climate and compared current and past thermal performance in relation to recent climate change. We concluded that overall maximum feeding rates and TPCs differed among *Colias* populations and species, suggesting local adaptation to thermal environment. Our results show population differences in TPCs that differ from those found by Sherman and Watt (1973) for two of the populations (Sacramento Valley, CA and Montrose Valley, CO). We expanded the experiment and included both higher elevation (Gunnison, CO) and variable season (Chapel Hill, NC) populations.

Larvae from Sacramento Valley, CA exhibit different thermal adaptation as they had a much lower F_{max} and T_{opt} from the other populations. These larvae also had the largest B_{80} indicating that they are likely temperature generalists and can achieve high performance at a wide variety of temperatures. Notably, one environmental difference between the Sacramento Valley and other populations is length of growing season. In contrast to the limited growing seasons for the other populations, which are punctuated by winter, the larvae from the Sacramento Valley are able to feed almost year-round thereby relaxing selective pressure on the shape of the TPC. Additionally, *C. eurytheme* from Sacramento Valley, CA are able to feed throughout the day and night unlike the other populations that are to feeding only during the day when temperature are high enough. As mentioned in the methods it is possible that the Chapel Hill, NC population

may include some *C. philodice* and *C. eurytheme* hybrids. However we did not see any detrimental fitness effects that could have been caused by hybridization. In addition, we were examining thermal sensitivity, which should not be affected by hybridization.

Despite living in areas with cooler mean annual temperatures, the *C. eriphyle* populations had high F_{max} and T_{opt} values compared to the *C. eurytheme*. In addition the T_{opt} was nearly as high as T_{air} or Montrose Valley and above T_{air} for the Gunnison, CO population indicating that the larvae are capable of feeding at higher temperatures than they typically experience. Due to shorter growing seasons and greater diurnal temperature variation, feeding is restricted to daytime during the summer months.

Colias eriphyle larvae from Gunnison, CO are able to continue feeding at temperatures well past their T_{opt}. These temperatures are generally considered stressful for *Colias* larvae (Sherman and Watt 1973). However, the negative effects may not have been measurable over the short exposure time. Other caterpillars have shown non-zero consumption rates past their thermal range as well. For example, *Pieris rapae* caterpillars from Seattle, Washington showed short-term (2-6 hours) maximal growth rates at 35°C despite optimal long-term growth occurring at 30.5°C (Kingsolver, 2000).

The *C. eriphyle* larvae from Gunnison, CO have a T_{opt} about 6°C higher than the larvae from Montrose Valley. This is contrary to other TPC studies showing that as elevation increased, T_{opt} decreased in neo-tropical high elevation frogs (Navas 1996). One possibility is that populations at higher elevations are strongly limited by the length of the growth season, resulting in countergradient patterns of growth across the elevational gradient. There is evidence of countergradient variation in growth across latitudes for some insects and other ectotherms (Arnett and Gotelli 1999, Van Doorslaer and Stokks

2005). For example, Conover and Present (1990) found that that high latitude Atlantic silverside fish (*Menidia menidia*) are adapted not to lower temperatures, but to rapid growth and consumption during the brief time of year when temperatures are high. A similar trend may be occurring in Gunnison, CO with the larvae adapted to feeding rapidly during shorter exposure to high temperatures rather than feeding slowly across a broader range of temperatures.

It is also possible that the larvae in Gunnison, CO are actually experiencing warmer body temperatures than the larvae in other populations due to the higher elevation larvae receiving more solar radiation. Larval body temperature has not been measured in the field although temperatures for the adult butterflies have not shown any difference in body temperature between populations (Kingsolver 1983a).

Climate changes and population responses

Mean air temperatures at these study sites have moderately changed from 1961-1971 to 2001-2011 however there has been a much larger increase in temperature variability. Previously, the temperature density at both Sacramento Valley, CA and Montrose Valley, CO was unimodal, but the current temperature data shows more variability. There has been an increase in the density of higher temperatures (above 28°C) from 2001-2011 versus from 1961-1971. The frequency of air temperatures above 28°C has increased two-fold in Sacramento Valley, CA and more than four-fold in Montrose Valley, CO. In general, the Rocky Mountains in Colorado are seeing a higher degree of climatic warming than other parts of the continental North America (Ray and Averyt, 2008).

The temperatures recorded at each weather station are the maximum and minimum T_{air} for the day measured 2m above ground level. These temperatures may not represent the temperatures that larvae would experience while foraging on plants in the field. Adult *Colias* butterflies require a body temperature of 30-40°C to achieve flight and do so despite experiencing a much lower T_{air} (Kingsolver, 1983). Near ground temperatures can be warmer than T_{air}, particularly under the high radiative conditions found at higher elevations, and may account for some of the variation in T_{opt} and temperature density.

This greater incidence of warm temperatures may be leading to the increased feeding at higher temperatures. However, the response to hotter temperatures varies between populations. The *C. eurytheme* larvae from the Sacramento Valley, CA have broadened their TPC to include a new range of temperatures over which they can feed as seen by the increase in B_{80}, whereas *C. eriphyle* from the Montrose Valley, CO have retained a similar TPC shape as seen by similarities in ρ and σ, despite increasing T_{opt} and thereby shifting the entire TPC to account for the hotter temperatures (Huey and Kingsolver 1993).

Methodological differences cannot explain all of the differences we saw in the past versus current experiments. Despite differences in how feeding rate was assessed between the past experiment and our own, we saw feeding in our experiment at both high and low temperatures where the previous feeding rate was zero. This underscores that despite some methodological differences, phenotypic changes in TPCs have occurred in these populations. In our current experiment the larvae were allowed to acclimate for 15 minutes prior to the feeding trial. It is unclear if larvae in the previous experiment were

allowed to acclimate at their experimental temperature before feeding. Acclimation in theory could lead to higher feeding rates and a greater B_{80} at all temperatures. Indeed, we saw an increase in the B_{80} for the *C. eurytheme* larvae, however this effect was not universal, and we did not see the same effect with *C. eriphyle.* Therefore, it is not differences in our acclimation that affected the differing B_{80} and T_{opt} between past to current experiments.

This study is among the first to show population changes in physiological performance in response to recent climate change, although previous theoretical work has predicted such changes (Visser 2008, Skelly et al. 2007, Hoffmann and Sgrò 2011). While previous work has highlighted adaptation to seasonal timing, specifically photoperiodic cues (Bradshaw and Holzapfel 2001), our work suggests that rapid adaptation to changing thermal regimes may also be an essential mechanism. Future work could explore whether similar shifts in thermal optima exist during egg and larval development and whether such adaptations will represent a general mechanism for rapid adaptation to climate change.

FIGURES

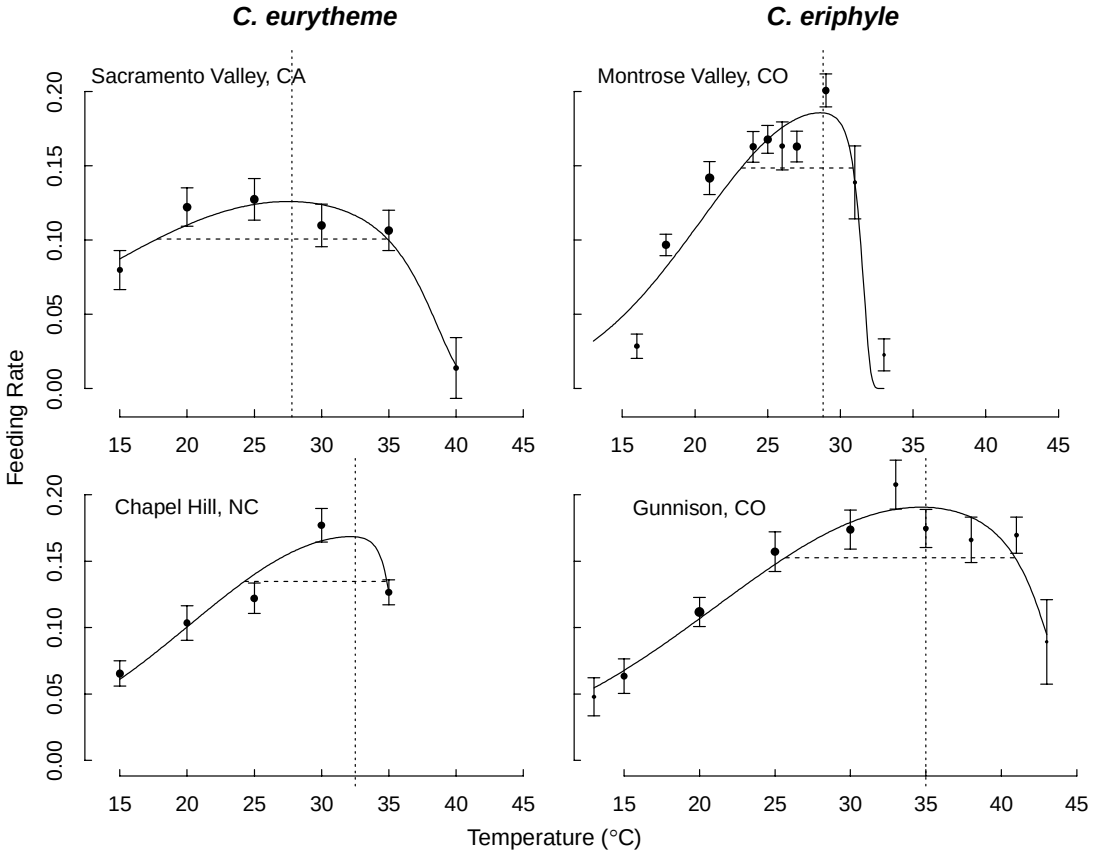

Figure 2.1: The thermal performance curves for feeding rate (mean +/- SE of ln(final mass/initial mass)/time) between the four populations. See the methods for an explanation of how changes in mass reflect short term feeding rate versus growth. The curve is the fit of the Frazier (2006) TPC model. The dotted horizontal line is B_{80}. The vertical line indicates T_{opt}. The size of the points is proportional to the number of larvae measured at each temperature.

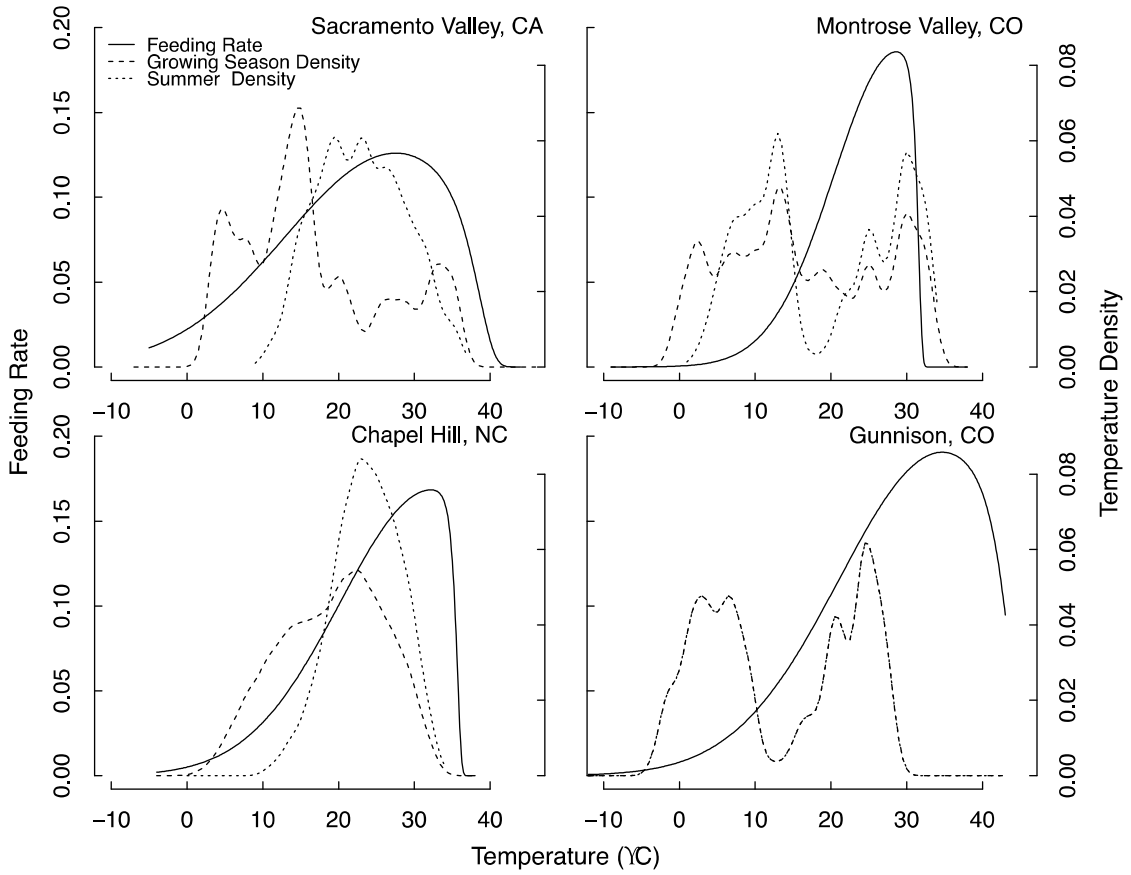

Figure 2.2: Feeding rate (solid line) and T_{air} during the growing season for each population. The temperature density is depicted for both the appropriate growing season (dashed) and the summer months (June 1-September 30, dotted). The growing season and summer months are the same for Gunnison, CO.

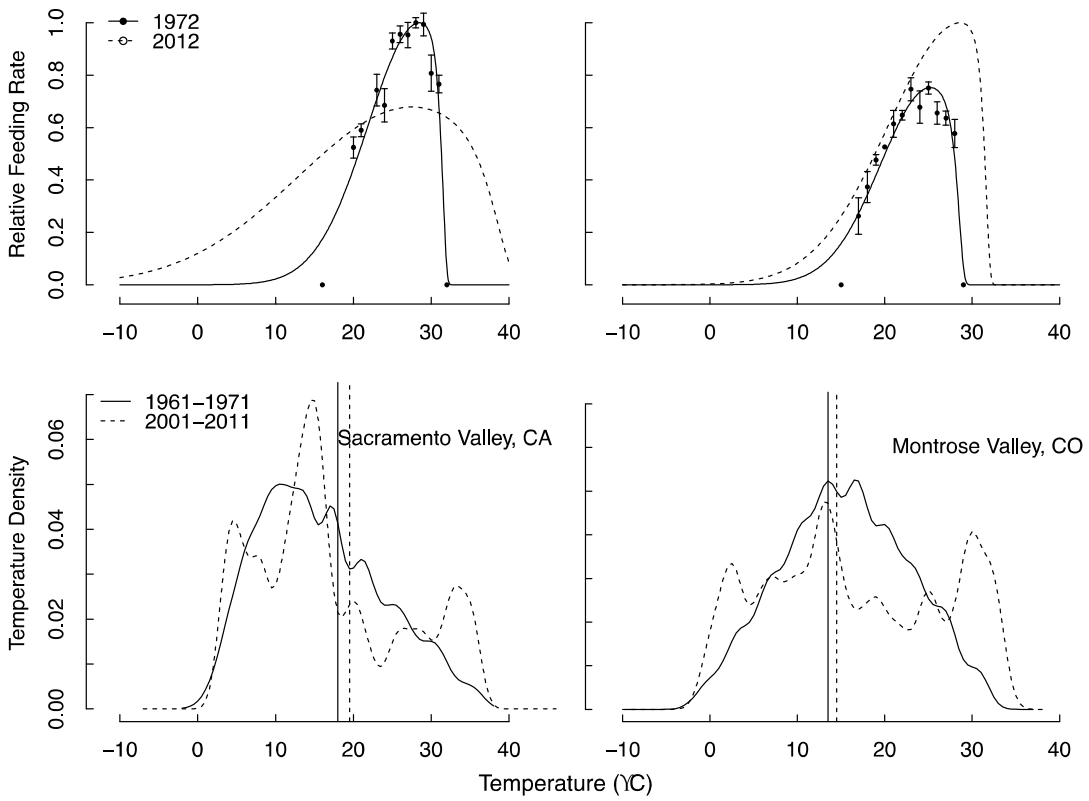

Figure 2.3: Historical comparison of larval feeding rate and temperature density during the growth season for *C. eriphyle* and *C. eurytheme*. The solid line designates data from the past and the dashed line is current data. The points are measured feeding rates in the Sherman and Watt 1972 experiment (mean +/- SE). Relative feeding rate is calculated by standardizing the highest feeding rate for each year to one. The vertical lines indicate mean temperature.

TABLES

Table 2.1: Results of ANOVA for the effects of temperature (T) and population (P) on feeding rate in *C. eriphyle* and *C. eurytheme*.
Note that the population term indicates differences among populations in overall rate of feeding, and interaction terms indicate differences between populations in thermal sensitivity of feeding rate. Standard deviation σ, for random effects are family=0.012 and individual within family= 0.015.

Parameter	**DF**	**F-value**	**p-value**
T	1	53.14	**<.0001**
T^2	1	130.16	**<.0001**
T^3	1	0.26	0.61
P	3	9.39	**0.0001**
T:P	3	15.19	**<.0001**
T^2:P	3	12.61	**<.0001**
T^3:P	3	1.60	0.19

Table 2.2: Parameter estimates for the thermal performance curve (+/- SE).
Where F_{max} is maximum feeding rate, T_{opt} is optimal temperature, and ρ and σ determine the thermal sensitivity of feeding at temperatures above and below T_{opt}, respectively. We also computed thermal breadth B_{80} as the temperature range for which the feeding rate is 80% of the maximal rate F_{max} *T_{opt} was given for the Chapel Hill, NC population in order for the model to converge. This value was chosen by finding the lowest residual error. Note: F_{max} for the 1972 experiment was measured as mm^2 leaf eaten/sec whereas for the 2012 experiment is was ln(final larvae mass/initial larvae mass)/time.

Species	**Population**	**F_{max}**	**T_{opt}**	**ρ**	**σ**	**B_{80}**
C. eurytheme	Sacramento Valley, CA- 19m	0.13 +/- 0.01	27.8+/- 2.1	0.54+/- 0.12	0.002+/- 0.001	17.1
C. eurytheme	Chapel Hill, NC- 148m	0.17+/- 0.01	32.5*	1.87 +/- 0.22	0.003+/- 0.0008	10.6
C. eriphyle	Montrose Valley, CO-1633m	0.19 +/- 0.02	28.8 +/- 2.8	2.14 +/- 3.10	0.007 +/- 0.004	7.7
C. eriphyle	Gunnison, CO- 2347m	0.19 +/-0.01	35.0 +/- 1.5	0.67 +/- 0.16	0.003+/- 0.0006	15.2
C. eurytheme	*Sacramento Valley, CA (1972)*	*0.27 +/- 0.01*	*28.6 +/- 0.6*	*2.06 +/- 0.47*	*0.011 +/- 0.003*	*6.6*
C. eriphyle	*Montrose Valley, CO (1972)*	*0.20 +/- 0.01*	*25.3 +/- 0.6*	*1.84 +/- 0.37*	*0.015 +/- 0.003*	*6.2*

Table 2.3: Results of ANOVA for the effects of temperature (T), population (P), and year (Y) in the historical comparison between 1972 and 2012 of *C. eriphyle* and *C. eurytheme* feeding rates.

Parameter	**Df**	**F-value**	**p-value**
T	1	0.07	0.79
P	1	2.48	0.12
Y	1	326.29	**<2.20E-16**
T^2	1	152.75	**<2.20E-16**
T^3	1	0.30	0.58
T:P	1	2.70	0.10
T:Y	1	13.31	**0.0003**
T^2:P	1	27.75	**1.76E-07**
T^2:Y	1	15.46	**9.13E-05**
T^3:P	1	0.96	0.33
T^3:Y	1	2.99	0.08

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CHAPTER 3: GROWTH, DEVELOPMENTAL, AND STRESS RESPONSES OF *COLIAS* LARVAE TO REPEATED EXPOSURE TO HIGH, SUB-LETHAL TEMPERATURES

The range of temperatures over which an organism can operate is often characterized by a thermal performance curve (TPC) (Huey & Stevenson 1979). Thermal performance curves have a particular shape with performance increasing gradually to the optimum and then rapidly decreasing at temperatures above the optimum. As the temperatures increases, ectothermic organisms reach their critical thermal maximum (CT_{max}) where the organism stops functioning and prolonged exposure can cause death. Upper thermal limits of terrestrial organisms do not vary consistently with latitude (Addo-Bediako, Chown & Gaston 2000; Sunday, Bates & Dulvy 2011) although thermal optima (T_{opt}) generally decrease with increasing latitude (Huey & Kingsolver 1993; Cunningham & Read 2003; Sun & Friedmann 2005). These contrasting environmental patterns for upper thermal limits and thermal optima make performance at temperatures above the optimum but below between the thermal maximum of particular interest.

We know there are differences in how TPCs relate to physiological traits between temperate and tropical species and across latitude (Cunningham & Read 2003; Deutsch *et al.* 2008; Sunday *et al.* 2011; Nilsson-Örtman *et al.* 2012). However, we do not know much about how these differences in TPCs will affect growth and development across elevations or between populations (Berven 1982). To explore this we used the sulphur butterfly, *Colias eriphyle,* from two sites in Colorado that differ in elevation as well as

environmental temperature regimes. The *C. eriphyle* larvae from these populations also differ in their TPCs for feeding rate (Figure 3.1) (Higgins *et al.* 2013). Here we investigate how different TPCs for short term feeding rates can influence long-term growth and development and whether sub-lethal temperatures are sufficient to elicit heat stress responses.

The heat shock response often determines the maximum temperatures of TPCs (Feder & Hofmann 1999), but we do not know at what temperatures this response initiates for many species, including *C. eriphyle*. Heat shock proteins are small chaperones that prevent protein misfolding during stress, however their production comes at the cost of growth and other cellular processes (Krebs & Feder 1997). We measured the expression level of heat shock protein 70 (*hsp70*) before, during, and after the heat treatment to understand how expression changes with time. We also determine whether expression levels of *hsp70* differ between the two populations. Previous studies with other organisms show differences in gene expression or protein levels for heat shock genes, including *hsp70,* across geographic gradients (White, Hightower & Schultz 1994; White *et al.* 1994; Tomanek & Somero 1999; Sagarin & Somero 2006).

We predict that the heat treatments should be more stressful for the larvae from Montrose Valley because of their lower optimal temperatures and upper thermal limits. Specifically we expect both of our heat treatments (high 33°C and high 38°C) to be stressful for the Montrose Valley larvae as the temperatures correspond to a feeding rate of zero. The feeding rate for Gunnison larvae is maximal at ~35°C and declines above 38°C. We predict that the Montrose Valley larvae will not be able to feed during the hottest parts of the three day heat treatments whereas the Gunnison larvae should be able

to continue feeding. Therefore we predict an overall decrease in the growth rate of Montrose larvae.

The time scale used to measure CT_{max} and upper thermal limits varies between studies, and typically an experimental organism only experiences a single exposure to high temperature during development. In this study we use repeated exposure to high non-lethal temperatures in order to understand both the short and long term effects of potentially stressful temperatures during development. This simulates the effect of heat waves that occur with extreme highs over a series of days. To examine the long-term effects, we measure mass and development time at each instar and to pupation. Based on short term TPCs for feeding rate we are also able to estimate and predict growth rate to pupation and compare our prediction with our results for the two populations with different TPCs for feeding. To study the short-term effects of heat exposure we look at *hsp70* expression levels before, during, and after the treatments. The fitness costs associated with the heat shock response may provide a partial explanation for some of the long-term fitness effects.

Methods

Study system

Colias eriphyle occurs in open habitats in the western US, and occupies elevations between 1,400-2,900m in western Colorado. The larvae have five larval instars and undergo a facultative winter diapause during the 3rd instar. The larvae feed on plants in the *Fabaceace* family, including *M. sativa* (alfalfa), *Vicia* (vetch) *spp., and Trifolium* (clover) *spp*.). We sampled *C. eriphyle* females from alfalfa (*Medicago sativa*) fields located in the Montrose Valley, CO (N38.62, W108.02, 1,633 m) and from a county park

with meadows including vetch (*Vicia)* and clover (*Trifolium)* near Gunnison, CO (N38.56, W106.94, 2,347 m). These two study sites have different growing seasons, which account for variation in larval development, adult flight time, and the number of generations per year (voltinism). In the Montrose Valley, CO the growing season can start as early as April and continue through October resulting in 3-5 generations of *C. eriphyle* per year whereas in Gunnison, CO, the growing season starts in June and continues through September, resulting in two generations of *C. eriphyle* per year (Higgins et al. 2013).

Growth and development experiments

Adult female butterflies were collected from each site and shipped overnight to the University of North Carolina at Chapel Hill. The female butterflies were kept in cages at greenhouse conditions (~26°C) under natural light. Females were fed 10% honey water solution by moistened sponge changed daily, and were allowed to oviposit on potted *Vicia villosa* in the greenhouse. Eggs were removed each day and placed in environmental chambers (Percival 36VL, Geneva Scientific, WI, USA) maintained at 21-29°C (average of 25°C) on a 14L:10D photoperiod where larvae were given leaves of *V. villosa ad libitum*. Larvae were reared individually and scored daily for age and instar. Upon entering the second instar, approximately 60 larvae from each population (180 larvae total from each population) were randomly placed into one of three temperature treatments: a medium heat treatment, 21-33°C, ramping from 21°C at 3:00 to 33°C at 15:00, holding at 33°C for an hour and then ramping steadily back to 21°C at 3:00 the next day; a high heat treatment, 21-38°C ramping from 21°C at 3:00 to 38°C at 15:00, holding at 38°C for an hour and then ramping steadily back to 21°C at 3:00 the next day;

or the control group which went back into the rearing chamber ramping from 21°C at 3:00 to 29°C at 15:00, holding at 29°C for an hour and then ramping steadily back to 21°C at 3:00 the next day. These temperatures were chosen based on the differences in mean TPCs for larval feeding rates between the two populations (Higgins *et al.* 2013): in particular, the mean optimal temperature for feeding is lower for the Montrose Valley population (T_{opt} = 28°C) than for the Gunnison population (T_{opt} = 35°C) (Fig 3.1). Because of these differences, we predicted that both the 33°C and 38°C treatments should be stressful for Montrose Valley, CO larvae. The Gunnison, CO larvae should not be stressed at 33°C and should face mild stress at 38°C. However, neither treatment should be lethal or cause permanent damage to the larvae.

Each larva was kept in their respective temperature treatment for three days and then returned to the control rearing conditions for the duration of the experiment. Age at each instar, mass at each instar beginning at the 3rd instar, overall growth rate to pupation, and development time were recorded. Development time, pupal mass, and growth rate were analyzed with population and temperature as fixed effects and sib-family (mom) as a random effect in linear mixed effects models using the *nlme* package (Pinheiro *et al.* 2014).

Expression of hsp70

RNA was extracted using Qiagen RNeasy kits (Qiagen, Valencia, CA) from whole larvae at four time points during the three-day heat treatments (10 biological replicates, with 3 technical replicates each) (at 0, 24, 72, and 96 hours) (Fig 3.2). Extracted RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA) with random primers. *Manduca*

sexta primers for *hsp70* (F: 5'-GTGCTGACCAAGATGAAGGA- 3', R: 5'-CGCTGTGAGTTGAAGTA-3') and for 18S rRNA (F: 5'-CAGCACATCTTAGGGCATCAC-3', R: 5'- CAACTCACTGGCGACGTATTA-3'), which was used to normalize the expression levels of *hsp70*, were used in the PCR of the cDNA. The PCR product was sequenced and *Colias* specific *hsp70* (F: 5'-CCAGTAACAACCTTGGCAAAC-3', R: 5'- CTGTGAGTCGTTGAAGTACG- 3') and 18S rRNA (F: 5'- CTCATCTCGTGCGGCT -3', R: 5'- GTAATCAACTCACTGGCGA -3') primers were designed and used for qPCR. The qPCR was conducted with the SYBR Green FastMix (Quanta Biosciences, Gaithersburg, MD) on a Biorad CFX96 thermocycler (Bio-Rad, Hercules, CA). PCR was initiated with a Taq activation step performed at 95°C for 10 min followed by 40 amplification cycles of a 95°C denaturation step for 2 sec and a 72°C combined annealing-elongation step for 10 sec. The data was analyzed using the Pfaffl method (Pfaffl 2001). Expression of *hsp70* was relative to 18S rRNA and calibrated to the control (high 29°C) larvae from Montrose Valley. Linear mixed effects models were used to analyze differences in relative expression ratio among treatments and time points with sib-family as a random effect and time, temperature, and population as fixed effects. RNA from the Gunnison larvae was only collected at 0 and 96 hours due to a laboratory accident that destroyed many of the samples. All data were analyzed in R (v 3.1.1).

Results

Growth and development

For both populations, the 33 and 38°C heat treatments decreased mean development time to 3rd, 4th, and 5th instars (Fig 3.3). However, there were no

significant effects of heat treatment ($F_{(2,348)}$=1.76, p=0.17), population ($F_{(1,23)}$=0.37, p=0.55) or their interaction on mean development time to pupation ($F_{(2,348)}$=0.41, p=0.66) (Fig 3.4). This suggests that the heat treatments accelerated development during the middle (2^{nd} - 4^{th}) larval instars, but these effects disappeared by the end of larval development.

For pupal mass, there were significant effects of treatment ($F_{(2,330)}$=6.34, p=0.04) and population ($F_{(1,22)}$=105.6, p<0.0001) as well as an interaction between treatment and population ($F_{(2,330)}$=4.8, p=0.009) (Fig 4). Increasing treatment temperatures reduced mean pupal mass for the Montrose Valley population, but not for the Gunnison population (Fig 3.4).

We also calculated growth rate to pupation and detected significant effects of population ($F_{(1,22)}$=19.9, p=0.0002) and of the interaction between treatment and population ($F_{(2,329)}$=1.0, p=0.37), but treatment alone was not significant ($F_{(2,329)}$=1.0, p=0.35) (Fig 3.4). Relative to the control group, mean growth rate for the Montrose Valley population was slightly lower for the 33°C treatment and much lower for the 38°C treatment (Fig 3.4). In contrast for the Gunnison population, the mean growth rate increased in the 33°C treatment, but decreased in the 38°C treatment (Fig 3.4).

hsp70 expression

For the Montrose Valley larvae, the relative expression levels of *hsp70* were not affected by temperature ($F_{(1,53)}$= 3.36, p=0.072), but did vary significantly over time ($F_{(1,53)}$= 7.35, p=0.01), and there was no significant interaction of time and temperature treatment ($F_{(1,53)}$= 2.20, p=0.144) (Fig 3.5). The expression levels were highest 24 hours

after being placed into the treatment (106 hours since hatching) and then went back down to pre-exposure levels.

When comparing the relative expression levels of *hsp70* between Montrose Valley and Gunnison before and 24 hours after the onset of the treatment (106 hours since hatching) there was a significant effect of population ($F_{(1,8)}$= 6.9, p=0.03), but there was no significant effect of temperature ($F_{(1,19)}$= 1.11, p=0.31), nor was there an interaction between the two ($F_{(1,19)}$= 0.67, p=0.42). The expression levels of *hsp70* at 0 and 24 hours were higher in the larvae from Gunnison compared to those from Montrose Valley.

Discussion

We found that the effects of high sub-lethal temperatures on larval growth and development differ between *Colias* populations, such that the lower elevation Montrose Valley is more susceptible to the stress of the heat treatments than the higher elevation Gunnison. This may be due to the fact that Gunnison has a more variable climate overall, and therefore the larvae are able to deal with more stressful temperatures.

Growth and development

Previous work looking at ramping heat treatments during the egg stage of *Manduca sexta* showed that the high temperatures slowed down development in the early instars, but that effect was gone in the later instars and pupation (Potter, Davidowitz & Arthur Woods 2011). Conversely, we found the heat treatments decreased the age at each instar in the 2nd through 4th instars (speeding up development), but had no effect on overall development time to pupation (3.3). The substantially higher temperatures used in

the Potter, Davidowitz & Woods (2011) likely explains why they saw delayed development associated with a stress response where we saw accelerated development.

Generally, higher temperatures during development cause rapid growth but a smaller adult body size. This is commonly known as the temperature size rule (TSR) (Atkinson 1994) and it has been shown in many ectothermic species (Sibly & Atkinson 1994; Atkinson & Sibly 1997; Angilletta & Dunham 2003; Kingsolver & Huey 2008; Forster, Hirst & Atkinson 2011). In our experiments, the heat treatments sped up development time for both populations; however, the Montrose Valley larvae were smaller at each instar when exposed to the heat treatments relative to the control (Fig 3.3). This could be simply the TSR in that faster development correlates with smaller size, however the smaller body size at each instar was not seen in Gunnison. Montrose Valley larvae may have experienced stressful times when the larvae did little to no feeding (or gaining of mass). The ontogenetic growth model suggests that there are tradeoffs between growth of new tissues and maintenance of existing tissues (West, Brown & Enquist 2001). Montrose Valley larvae facing high temperatures may have used their energy to protect the tissues they had by slight upregulation of *hsp70* (Fig 3.5) versus continuing consumption and gaining mass.

As predicted, the Montrose Valley larvae were significantly smaller as pupae when in the heat treatments relative to their control counterparts. Larvae experienced similar overall average temperatures despite three days of variable heat treatments. In the high 29°C treatment the mean temperature throughout development was 25°C, in the high 33°C treatment the mean temperature was 25.4°C, and in the high 38°C treatment the mean was 25.9°C. These differences in mean temperatures are insufficient to account for

the large differences in pupal mass observed. This suggests that it was stress, not simply the TSR, reducing pupal masses in Montrose Valley. Starved damselflies exhibited compensatory growth by increasing development time and mass, but had a lower mass at emergence ((Stoks, Block & McPeek 2006)). Stress associated with the three heat treatment days had long term effects on overall fitness via reduced pupal size (Taylor, Anderson & Peckarsky 1998).

hsp70 expression

Overall *hsp70* expression was higher in Gunnison compared to Montrose Valley, even at 29°C. Gunnison is higher in elevation and overall experiences generally cooler yet more variable temperatures than Montrose Valley. In other systems *hsp70* levels have been observed to decrease (Dahlhoff & Rank 2000; Garbuz *et al.* 2003) or remain constant (Karl *et al.* 2009) with elevation. However, Healy (2010) found that the cooler, northern populations of killifish did have higher expression of *hsp70-2* and *hsp90* than the warmer southern populations (Healy *et al.* 2010).

Heat treatment did not alter *hsp70* expression in the Gunnison population. Gunnison larvae may have reached their maximum levels of expression for *hsp70*, however this seems unlikely as they do not exhibit common symptoms such as decreased growth and development (Krebs & Feder 1997). The larvae are able to continue feeding well past the temperatures used in the heat treatments (Higgins *et al.* 2013). Alternatively, the observed *hsp70* expression in Gunnison larvae may represent basal levels if our heat treatments were insufficient to cause stress. Perhaps the overall variability of the temperatures experienced in the field lead to higher performance at extremes. Examining *hsp70* expression levels at much higher temperatures, specifically above 38°C where they

begin to decrease feeding, would help resolve these results. In Montrose Valley the expression levels increase at 24 hours in the heat treatment and then decrease to their pre-heat treatment levels. This is consistent with other work highlighting the rapid induction of *hsp70* during a heat stress and then a decrease of expression levels back to normal levels (Dahlgaard *et al.* 1998; Tomanek & Somero 1999; Tomanek & Sanford 2003).

Many of the studies looking at *hsp70* expression use adults, whereas we measured expression levels in larvae. *Hsp70* expression in *Tenebrio molitor* beetles was much lower in larvae compared to adults (Lardies *et al.* 2014). Even work examining *hsp70* expression throughout adult ages have concluded that expression decreases with age to adulthood (Sørensen & Loeschcke 2002), further highlighting that different life stages and ages have can have different heat stress responses. *Colias* adults differ from larvae in thermal tolerance (Watt 1968; Kingsolver & Watt 1983; Kingsolver 1983). It is thus likely that the levels and even the temperature at which induction of *hsp70* occurs would be different in adults.

Exposure of *C. eriphyle* larvae to high sub-lethal temperatures early in development did not affect overall development time to pupation, but did cause differences in pupal mass and growth rate. The Gunnison population had higher *hsp70* expression levels overall compared to Montrose Valley, but the expression levels did not change before or during the heat treatment. This may signify that these heat treatments were not stressful enough to elicit a response, which does correlate with the TPC showing that Gunnison continues feeding past 38°C. Overall, we have shown that sub-lethal high temperatures have many varied effects on growth and development both in the short term and into adulthood, and that the effects can depend on population. This work

demonstrates the importance of considering responses to thermal stress at multiple time scales and throughout the life cycle.

FIGURES

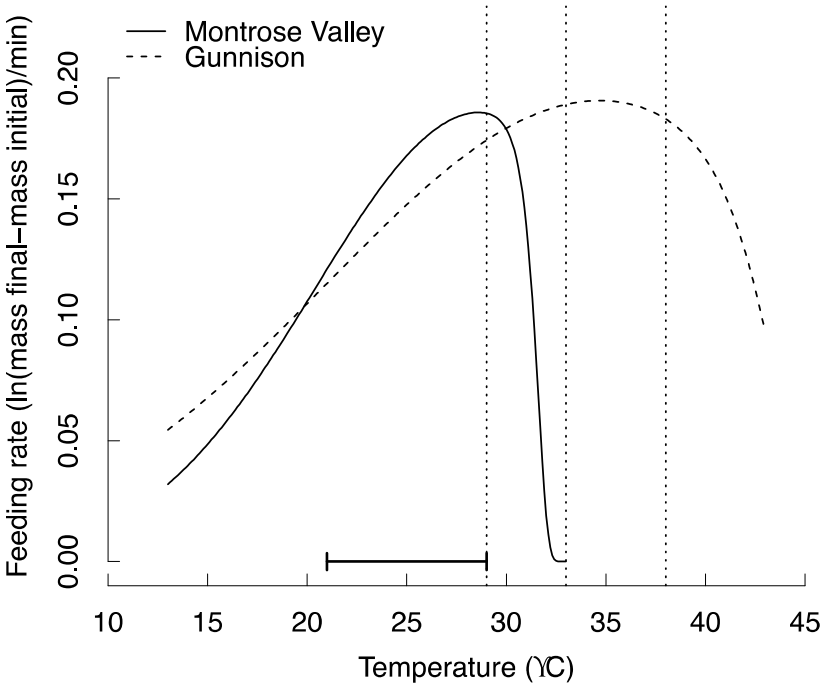

Figure 3.1: Short term feeding rate of 5th instar *Colias* larvae from Montrose Valley and Gunnison, CO. These feeding rates were used to determine the heat treatment temperatures (dotted vertical lines). The rearing conditions are indicated by the horizontal bar above the x-axis.

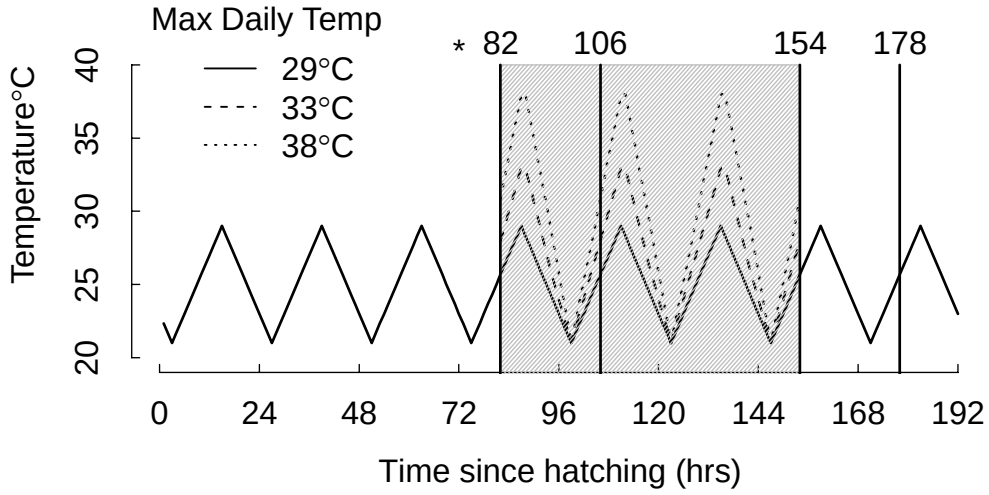

Figure 3.2: Temperature regime for the control (29°C) and heat (33°C and 38°C) treatments. The heat treatments (shaded box) began at the onset of the 2nd instar (indicated by star), which starts at approximately 3 days after hatching. The vertical lines indicate when larvae were sacrificed for the *hsp70* expression experiment.

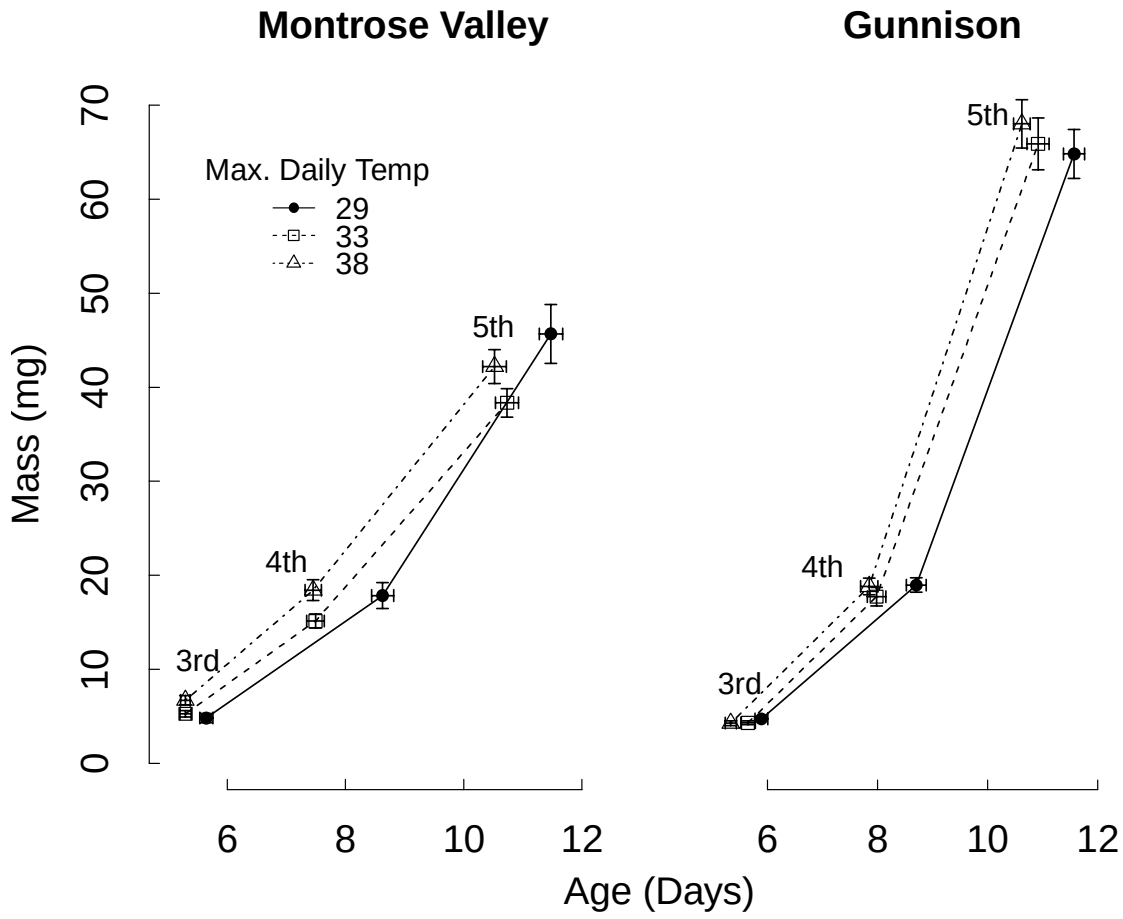

Figure 3.3: Mass and age from the 3rd to 5th instar for larvae in the control (high 29°C) or heat (high 33 or 38°C) treatments. Heat treatments occurred for 3 days (72 hours) following the onset of the 2nd instar.

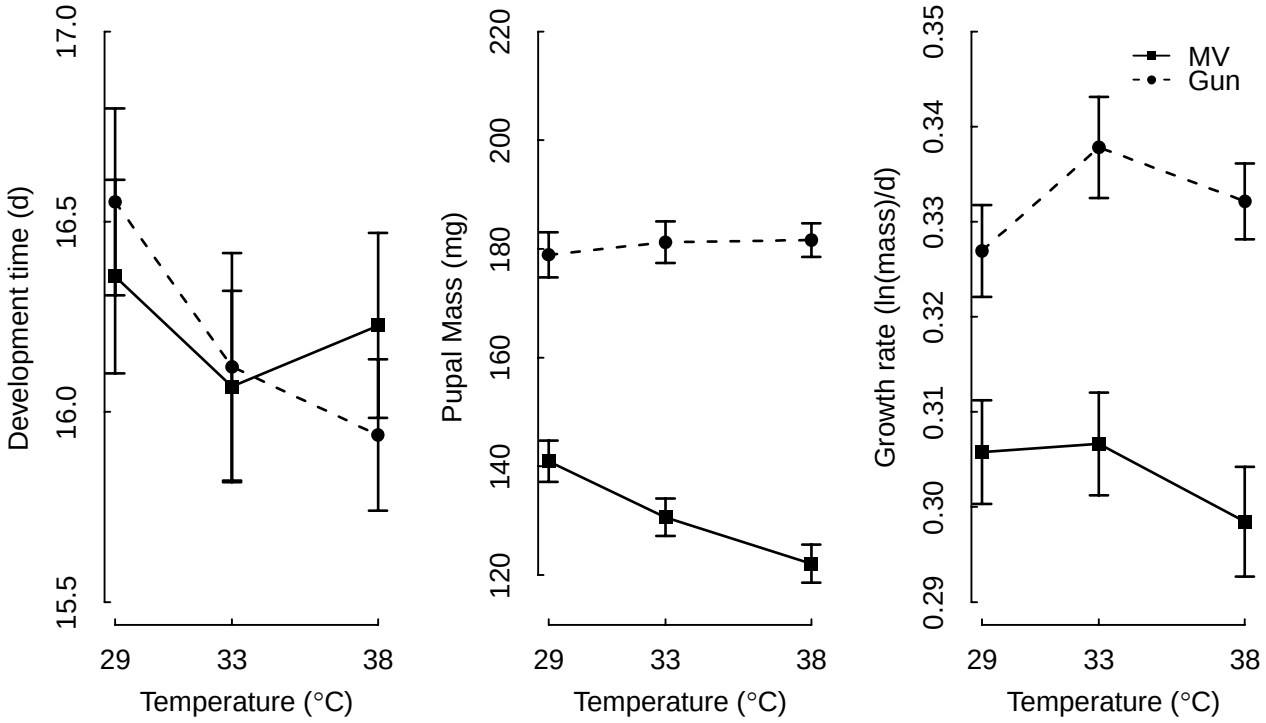

Figure 3.4: Development time to pupation, pupal mass growth rate for larvae from Montrose Valley (squares, dashed line) and Gunnison (circles, solid line).

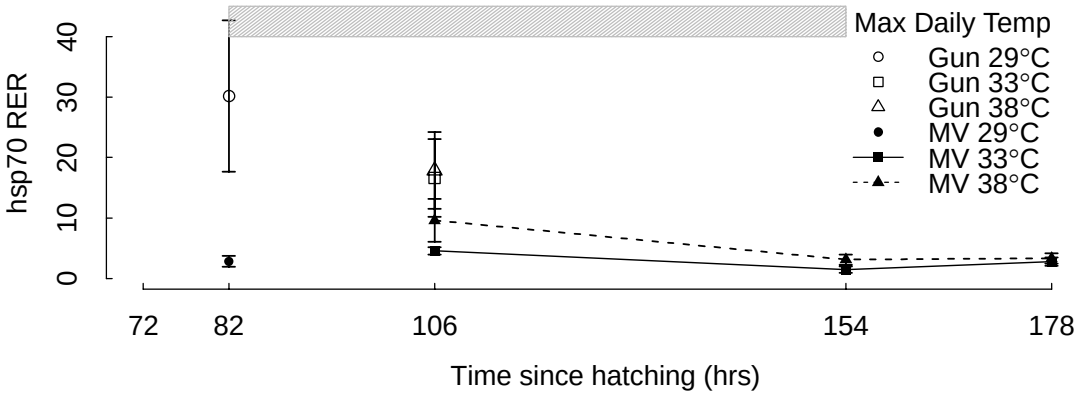

Figure 3.5: Relative expression levels of *hsp70* for larvae from Montrose Valley (solid shapes) and Gunnison (open shapes) at 33°C (solid line) and 38°C (dashed line). The shaded rectangle indicates the duration of the heat treatments. All expression levels were calibrated to the Montrose Valley control (high 29°C) levels.

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CHAPTER 4: THERMAL SENSITIVITY OF PUPAL LIFE HISTORY AND PLASTICITY OF ADULT MORPHOLOGY IN *COLIAS ERIPHYLE*

Introduction

For ectotherms, temperature affects nearly all of life's processes, and differing temperatures throughout development can have affect different fitness and performance traits. For insects that undergo pupation this sessile life stage can present unique thermal challenges (Kingsolver *et al.* 2011). Pupae cannot thermoregulate meaning they experience the temperature of their surrounding environment. Previous work looking at the thermal effects of pupation have shown that both high and low temperatures can cause decreases in survival and that temperature can also affect pupal development rate (Turnock, Lamb & Bodnaryk 1983; Lamb & Gerber 1985; Gotthard, Nylin & Wiklund 1994; Krebs & Loeschcke 1995; Tarone *et al.* 2011; Telles-Romero *et al.* 2011). As temperatures increase pupal development speeds up and pupae can emerge sooner, this may be beneficial as it decreases the amount of time that an organism is completely susceptible to predation (Evans *et al.* 2013). However, early emergence may mean that there is a mismatch of resources and there is the possibility of experiencing cold temperatures again due to a false spring (Bale *et al.* 2002). During pupation organisms can only lose mass as they use their resources (Fischer *et al.* 2005). While higher temperatures speed up pupal development extremely high temperatures may have the detrimental effect of increasing mass lost during pupation due to consumption of resources. This may create smaller, less fit adults (Kingsolver & Huey 2008).

In addition to pupal life history traits, temperatures during pupation can also affect adult traits. Phenotypic plasticity allows organisms to adapt certain traits to a variable environment. Climate variation, predator regime, and critical photoperiod are all environmental variables to which some organisms, including butterflies, have shown plasticity (Nylin, Wickman & Wiklund 1989; Berwaerts *et al.* 1997; Nylin & Gotthard 1998; Karlsson & Van Dyck 2005; Breuker, Brakefield & Gibbs 2007). This phenotypic plasticity is specifically called seasonal polymorphism when adults have different morphs that increase fitness during different seasons. The tropical butterfly *Bicyclus anynana* has two different wing patterning phenotypes, associated with different seasons (wet vs. dry) and predators during each season (Lyytinen *et al.* 2004). Phenotypic plasticity may be a way for organisms to mitigate the harmful effects of a changing climate (Przybylo, Sheldon & Merilä 2000; Réale *et al.* 2003; Charmantier *et al.* 2008) such as increasing temperatures, but only if the traits are sufficiently plastic and if the cues used in triggering plasticity are reliable.

For *Colias* butterflies, adult wing melanin is a plastic trait that determines flight activity and ultimately fitness, as increased flight time leads to more egg laying in females (Kingsolver 1983; Buckley & Kingsolver 2012). We know that there are elevational patterns in the degree of wing melanin (Ellers & Boggs 2002a; Stamberger 2006) but we have limited evidence on the degree to which this trait is plastic in regards to temperature. For the low elevation *Colias eurytheme* adult wing melanin is related to the photoperiod during pupation with longer light cycles leading to lighter wings (Hoffmann 1973; Hoffmann 1978). In his studies Hoffmann noticed that while *C. eurytheme* wing melanin was related to photoperiod, wing melanin in the higher elevation

C. eriphyle was not. Rather, wing melanin in *C. eriphyle* was related to the temperature experienced as a pupae, with higher temperatures producing butterflies with lighter wings (Hoffmann 1973; Hoffmann 1978). This is likely because temperature and photoperiod are not correlated in variable montane environments. Hoffmann's (1973, 1978) studies showed that there is some effect of pupal temperature on adult wing melanin, but his study did not examine if temperatures during the larval and pupal stage could also affect other adult traits.

In this chapter I focus on the effects of differing temperatures during pupation for two populations of *Colias eriphyle*. We know that there are differences in larval performance (feeding rate) between the two populations at different temperatures (Higgins *et al.* 2013), however at the adult stage there are no differences between populations in thermal sensitivity of adult flight (Watt 1968). This makes the pupal stage particularly interesting to study. *Colias eriphyle* pupate during the summer months attached to host plants. Unable to thermoregulate, they are subjected to the conditions on their host plant. I want to know how different pupal temperatures affect pupal survival, pupal duration, adult mass, and the plasticity of adult wing ventral hind wing melanin? I want to understand the effects of temperature on pupal life history traits in *Colias eriphyle* and I want to know whether these effects differ between two Colorado populations. Additionally, I aim to study if pupal and larval-pupal conditions affect adult plasticity in regards to wing melanin, and by comparing back to Hoffmann's (1973, 1978) work I want to know if that plasticity has changed over time.

Methods

Female adult *C. eriphyle* were shipped from the Montrose Valley and Gunnison, CO during the summers of 2011 and 2012. In 2011, females from Montrose Valley were caught in early July, and females from Gunnison were caught in early August. In 2012, females from Montrose Valley were caught in mid-July and females from Gunnison were caught in mid-May. The females were shipped overnight on ice to the University of North Carolina at Chapel Hill and placed in greenhouse conditions (~26°C, ambient light). Females were allowed to oviposit in the greenhouse on *Vicia villosa* and given ~20% honey water *ad libitum.*

Pupal life history traits- 2011 and 2012

Every day, eggs were collected and placed into growth chambers (Percival) and reared at a constant 25°C, at 14L:10D. The larvae were kept on potted *V. villosa* plants by maternal family and reared together until pupation. Upon pupation, the pupae were placed into vented plastic cups with moistened filter paper to prevent desiccation and a wooden popsicle stick that created a perch for newly eclosed butterflies to dry their wings. The pupae were randomly assigned and placed into a new chamber at 15, 20, 25, or 30°C (in 2011 20°C, 25°C, 30°C for both populations; in 2012 15°C, 20°C, 25°C, 30°C for Gunnison and 15°C and 30°C for Montrose Valley) for the duration of pupation to eclosion. Pupal mass, pupal development time, adult mass, and survival were recorded for each individual. All of the pupae were weighed 48 hours after pupation to prevent death from handling. Mass lost was calculated for each individual as pupal mass-eclosion mass.

Plasticity of wing melanin- 2011 and 2012

Pupal temperatures

The experimental set up was the same as in the pupal life history traits experiment. Upon eclosion, butterflies from all temperature treatments were frozen, dried, and kept at 0°C until spectrophotometer analysis.

Larval-pupal temperatures

After hatching the larvae were placed into chambers that ramped from 16-24°C (average 20°C) or 21-29°C (average 25°C) all under 14L:10D light/dark cycles. The larvae were reared individually on cut *V. villosa* in petri dishes and fed *ad libitum*. The larvae remained in their respective chambers for the duration of development (larval and pupal). Upon eclosion, butterflies from all temperature treatments were frozen until spectrophotometer analysis.

Spectrophotometer analysis

Dried, frozen adult butterflies were mounted using Duco Cement onto 2x2 inch card stock square. Reflectance was measured at 650nm using a Field Spec Pro (Licor) spectroreflectometer by selecting a 2mm area for analysis below the discal spot (Watt 1968; Hoffmann 1978) as a proxy for wing melanin. I used black flocking paper with a 2mm window cut out to select only the area being studied. Reflectance was corrected by accounting for the background reflectance of the flocking paper. Three reflectance measurements were collected and averaged for each individual. Reflectance was then converted to absorbance (1-reflectance) to compare to historical results. Low absorbance shows a lighter wing melanin, high absorbance shows darker wing melanin.

Statistics

All analyses were conducted using the R (v3.1.1) statistical program. All of the data was analyzed separately for each year due to overall differences in 2011 and 2012.

Survival was analyzed using binomial generalized linear mixed effects models using the *lme4* package with population, sex, and temperature as fixed effects and sib-family as a random effect (Bates *et al.* 2014). Pupal duration, mass lost, and wing absorbance were analyzed with population, temperature, and sex as fixed effects and sib-family (mom) as a random effect in linear mixed effects models using the *nlme* package (Pinheiro *et al.* 2014).

Results

Pupal life history traits

Survival was high across all treatments for both years. Overall, survival in 2012 was lower than survival in 2011. For survival in 2011, population ($z= 0.14$, $p=0.89$) and temperature ($z= -0.54$, $p=0.59$) were not significant nor was the interaction of population and temperature ($z=0.002$, $p=0.10$) (Figure 4.1). In 2012, population ($z=0.24$, $p=0.81$) and temperature ($z=0.78$, $p=0.44$) were also not significant, nor was the interaction ($z= -0.43$, $p=0.67$) (Fig 4.1).

Generally, as the temperature increased the difference in the mean duration of the pupal stage between the Gunnison and the Montrose Valley pupae decreased until 30°C where their pupal duration was similar (Fig 4.2). High pupal temperatures decreased pupal duration in both 2011 ($F_{1,108}= 676.5$, $p<0.0001$) and 2012 ($F_{1,63}= 300.6$, $p<0.0001$). In 2011, population ($F_{1,18}= 12.7$, $p=0.002$) was significant and the pupae from Gunnison spent more time as pupae than those from Montrose Valley (Fig 4.2A), however this effect was not found in 2012 ($F_{1,16}= 1.03$, $p=0.3$). In 2011, there was no significant difference between the sexes ($F_{1,108}= 3.5$, $p=0.06$), but in 2012 males spent less time as pupae than females ($F_{1,63}= 5.2$, $p=0.03$). In 2011, there was a significant interaction of

population and sex ($F_{1,08}$= 9.2, p=0.003), such that males from Gunnison spent more time as pupae than females from Montrose Valley, however there were no significant interactions in 2012 (Fig 4.2).

For mass lost there was no significant difference between populations in 2011 ($F_{1,18}$= 0.08, p=0.78) nor 2012 ($F_{1,15}$= 2.6, p=0.12) (Fig 4.3). In 2011, higher pupal temperatures led to more mass lost during pupation ($F_{1,101}$= 6.5, p=0.01), this effect was not seen in 2012 ($F_{1,61}$= 0.16, p=0.7). There was no difference between the sexes in regards to mass lost in 2011 ($F_{1,101}$= 0.01, p=0.91), but in 2012 males loss more mass than females ($F_{1,61}$= 4.7, p=0.03). There were no significant interactions in 2011, but and there was an interaction of temperature and sex such that temperatures increased the mass lost for males increased, but the mass lost for females decreased ($F_{1,15}$= 4.4, p=0.04) in 2012 (Fig 4.3B).

Plasticity of wing melanin

For wing absorbance at 650nm, females were darker than males in both 2011 ($F_{1,93}$= 29.3, p<0.0001) and 2012 ($F_{1,54}$= 6.9, p=0.01). Temperature, population, and all interactions were not significant in 2011 (Fig 4.4A), but in 2012 higher temperatures led to lower wing absorbance ($F_{1,54}$= 6.4, p=0.01), however there was no difference in absorbance between populations ($F_{1,15}$= 1.0, p=0.34) (Fig 4.4B). In 2012, as temperatures increased absorbance increased for the females and decreased for the males ($F_{1,54}$= 10.6, p=0.002). For Montrose Valley the females were darker than the males, but for Gunnison the males were darker than the females ($F_{1,54}$= 12.1, p=0.001) (Fig 4.4B). The largest differences in absorbance are between the 15°C and 30°C temperature treatments.

Changes in plasticity over time

The absorbance at 650nm appears to be variable from year to year, especially when combining males and females (Fig 4.5). In 2012, the wing absorbance was higher than in 1978, which was about equal to the wing absorbance in 2011.

Pupal temperatures versus larval-pupal temperatures

To compare the effects of the cycling treatment during larval and pupal period I looked at the 2011 Montrose Valley larvae reared at either a constant 25°C throughout larval and pupal development or a cycling 21-29°C temperature (average 25°C) for both larval and pupal development. I found that females were darker than males ($F_{1,43}$= 19.94, p=0.0001) and the animals kept in constant temperatures were darker than animals kept under the ramping conditions ($F_{1,43}$= 7.7, p=0.008) (Fig 4.6).

When comparing the different ramping temperatures in 2011 Gunnison was measured at a ramping 20°C treatment and Montrose Valley at both a ramping 20°C (16-24°C, average 20°C) and 25°C (21-29°C, average 25°C) treatment. Overall, the absorbance in 2012 was higher than in (Fig 4.7). In 2011, wing absorbance was higher at 20°C compared to 25°C, ($F_{1,64}$= 28.6, p<0.0001) and females were darker than males in both 2011 ($F_{1,64}$= 11.3, p=0.001) and 2012 ($F_{1,137}$= 37.8, p<0.0001) however there was no significant difference between populations in 2011 ($F_{1,9}$= 0.04, p=0.95) nor in 2012 ($F_{1,18}$= 0.12, p=0.73). Additionally, the interaction of population and sex was not significant in 2011 ($F_{1,64}$= 0.84, p=0.36) nor 2012 ($F_{1,137}$= 1.6, p=0.21) (Fig 4.7).

Discussion

Pupal life history traits

Similar to other life stages, temperatures during pupation have many variable effects on survival, body size, and development time. Survival was generally high

although the survival during 2012 was lower than in 2011. One of the key differences in rearing conditions between the years was that in 2012 the larvae were kept in Percival chambers that also contained *Pieris rapae* larvae (as part of a different experiment). Although each larva was reared independently in an individual, closed petri dish, and *Pieris* and *Colias* larvae were in separate sections of the chamber, this may have contributed to the greater mortality in 2012 due to viral disease being spread in the chamber (Fig 4.1). The pupal temperatures used were not lethal or stressful as seen by the high survival. However it would be interesting to see the pattern of survival as temperatures increased further especially given the fact that the two populations have different thermal tolerances as larvae (Higgins *et al.* 2013).

The population differences in mean development time are largest at cold temperatures and disappear as temperature increases (Fig 4.2). This pattern was similarly seen in the *Colias* larvae when reared at two different temperatures (see Chapter 5). In 2011 for Gunnison, the estimated developmental zero or the temperature at which development stops is 12.9°C, and the slope of the regression of the development rate over temperature is 0.012, which is also the rate of accumulation of degree days. In 2011 the developmental zero for Montrose Valley is 13.7°C and the rate of degree-day accumulation is 0.015. In 2012, for Gunnison the estimated developmental zero is 10.2°C and the rate of degree-day accumulation is 0.014. For Montrose Valley in 2012, the estimated developmental zero is 9.1°C and the rate of degree-day accumulation is 0.009. Gunnison typically has a longer pupal development time, but at 30°C the development times between the two populations are nearly identical. There appears to be a lower limit

on development time in that the pupae need at least 5-6 days, to complete pupal development.

Mass loss during pupation increases with increasing temperature, as reported in other species (Telles-Romero *et al.* 2011). In 2012 males lost more weight than females as temperature increased (Fig 4.3B). For *Pararge aegeria*, the speckled wood butterfly, males also tend to lose more mass during pupation despite having similar pupal development times (Gotthard *et al.* 1994).

Wing melanin

In agreement with previous studies (Ellers and Boggs 2002), mean wing melanin (absorbance) was greater in females than in males. . This is likely a fitness advantage for females who initiate flight at higher body temperatures than males (Watt 1968). The darker wings allow them to heat up and achieve flight at their higher required body temperatures. This could present future problems for *C. eriphyle* females as air temperatures increase due to climatic change they may be more likely to suffer the ill effects of overheating. It is interesting though that in my experiment I did not see an effect of pupal temperature on wing melanin in females from either 2011 and 2012. In females, body heating is adaptive for maturation of eggs as well as for flight (Ellers & Boggs 2002, 2004), however females with higher degrees of melanin on their ventral wings obtained less matings than females with less melanin (Ellers & Boggs 2003). All of these factors suggest that the level of wing melanization in *C. eriphyle* females is a complex trait controlled by many factors.

Plasticity of wing melanin

In each variable measured I saw differences between the two years when I conducted the experiment. Although the mothers were caught during different times of the flight season, other studies have shown that maternal effects on wing melanin are negligible at best (Kingsolver & Wiernasz 1991; Ellers & Boggs 2002; Chaput-Bardy *et al.* 2014), suggesting that the differences I saw were likely due to the stressful conditions of viral disease. However, the disease in the chambers may have influenced wing melanin. Insect injury and infection triggers the phenoloxidase cascade (Cerenius & Söderhäll 2004) which can stimulate melanin pigment production and this may be the cause of the increased absorbance wing melanin from 2012 individuals. Freitak *et al.* (2005), found that *Pieris brassicae* given an immune challenge during pupation had darker and larger wing spots as adults. It is possible that the higher wing absorbance in 2012 may be due to concurrent infections and disease from crowded rearing conditions.

Historical changes in mean and plasticity of wing melanin

Ventral hindwing melanin does affect thermoregulation and flight performance of adult butterflies and there is a balance between having enough melanic scales to achieve a body temperature required for flight and having too many melanic scales and risking the detrimental effects of overheating (Watt 1968; Kingsolver & Watt 1983; Kingsolver 1983). I did not see any difference in wing absorbance when comparing my data to Hoffmann's previous work in the 1970s although field temperatures have changed in the past 40 years and we have seen differences in larval performance across temperatures (Higgins *et al.* 2013). This suggests that overall there have not been changes in the level of plasticity or the plastic response to temperature in these *C. eriphyle* populations.

Pupal temperatures versus larval-pupal temperatures

None of the work done on *Colias* wing melanin so far has determined whether it is mean pupal temperature or a single exposure to temperatures that influence adult wing melanin. In my comparison of the Montrose Valley larvae and pupae reared at either a constant 25°C or an average 25°C (ramping from 21-29°C) I found that the animals exposed to the ramping conditions had an overall lower wing absorbance than those reared under a constant 25°C (Fig 4.6). Although the average temperatures were the same, this perhaps suggests that the daily exposure to higher temperatures may be driving the lower wing melanin in the ramping conditions.

The effects of cycling temperatures (Fig 4.7) begin to elucidate this phenomenon, but more work comparing both constant and variable temperatures needs to be done. Additionally, there may be an undetected effect of larval temperature on wing melanin, however further studies in this area still need to be completed.

The highly variable nature of wing melanin also brings up many questions with temperature is a reliable versus just a good enough cue during pupation to predict future adult conditions. Further analysis of wing melanin levels in field caught bugs and correlation with temperatures experienced in the field could help elucidate this issue.

FIGURES

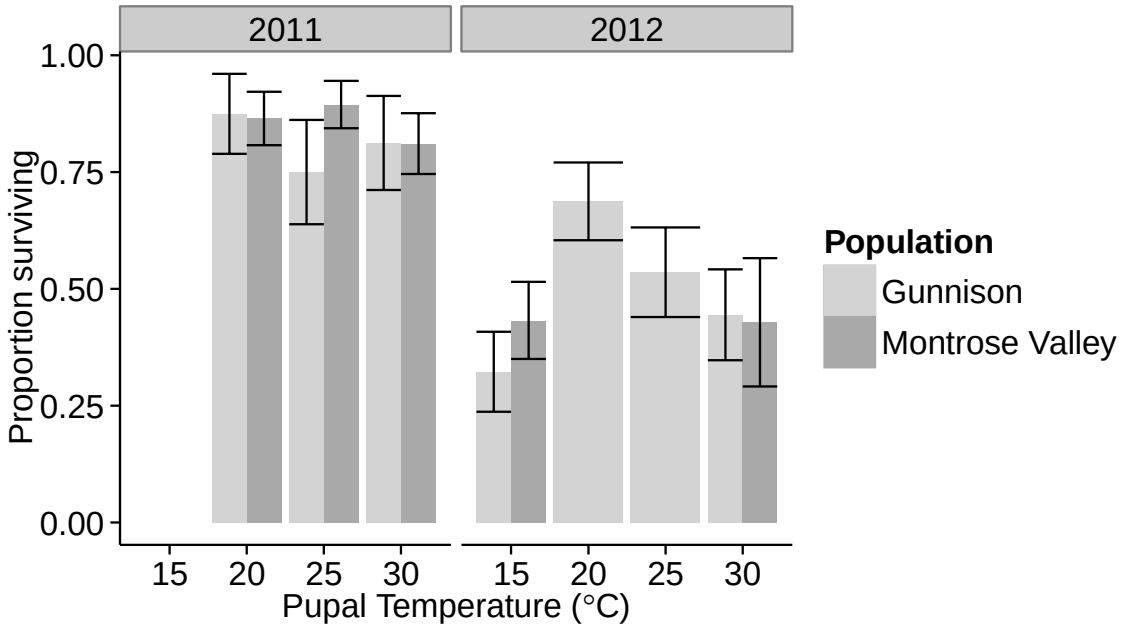

Figure 4.1: Proportion of surviving pupae in each temperature treatment for each year for Gunnison (light grey) and Montrose Valley (dark grey).

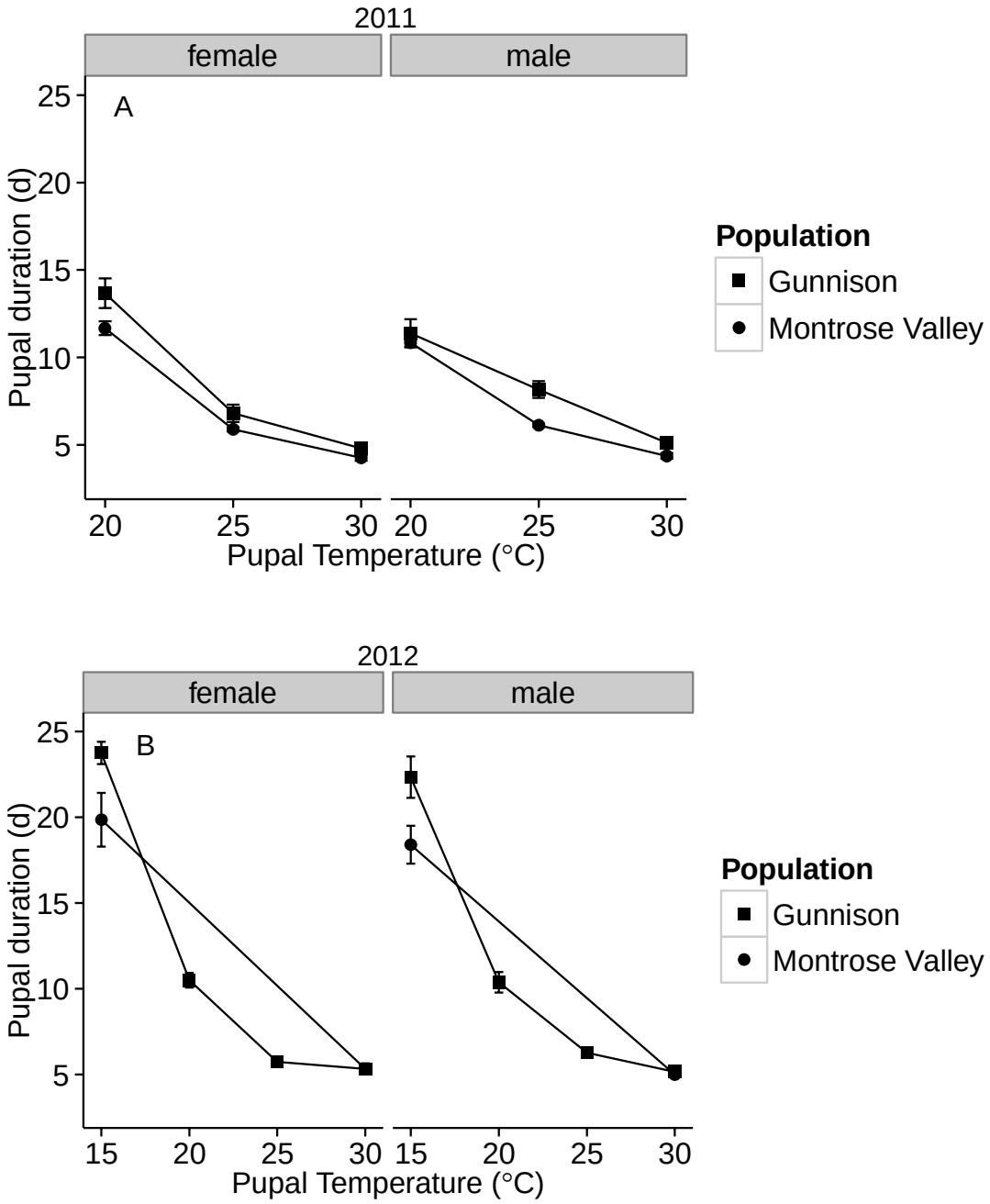

Figure 4.2: Duration of pupation by temperature (+/-SE) Circles are pupae from Montrose Valley, squares are pupae from Gunnison 2011 (A) 2012 (B).

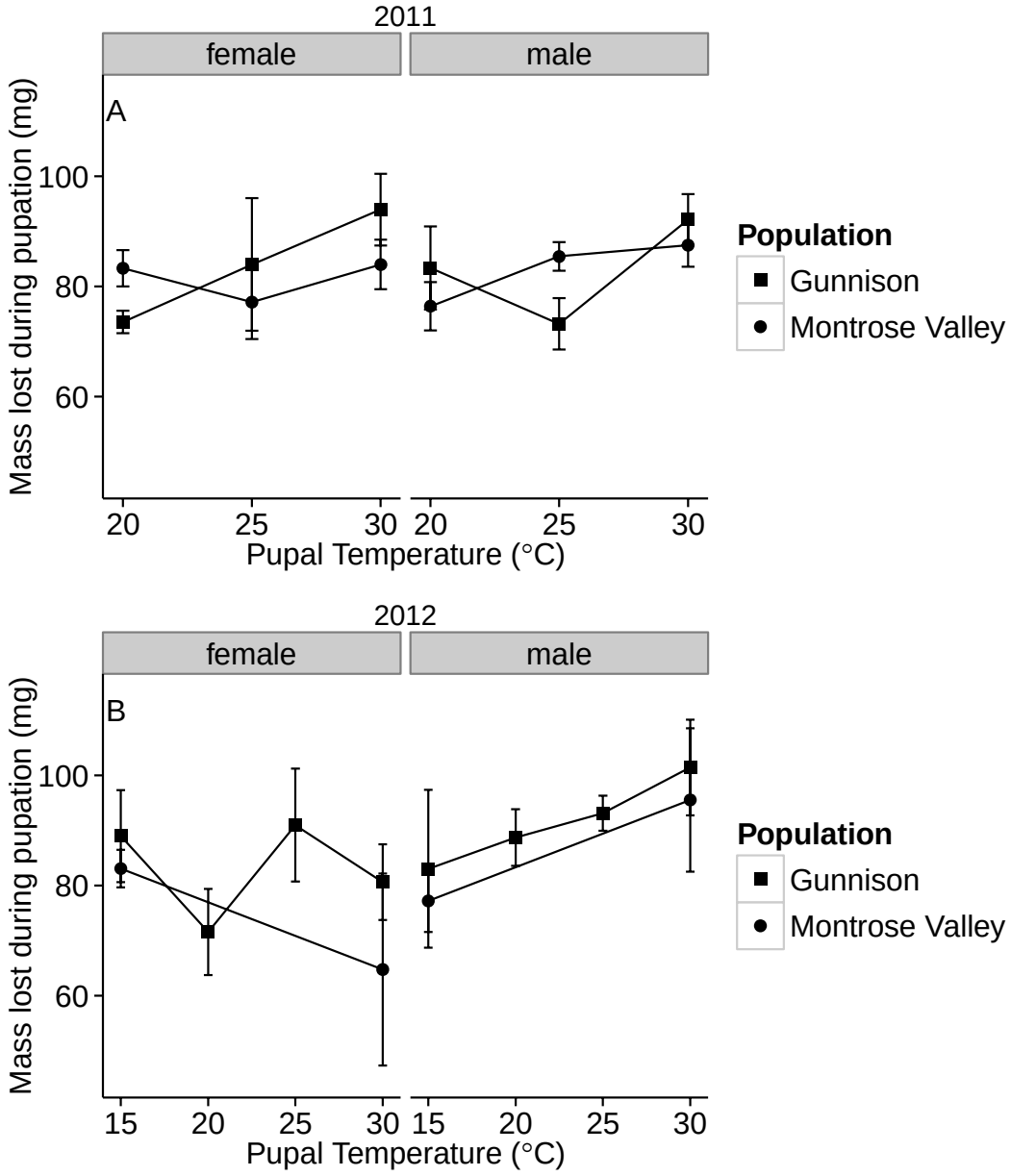

Figure 4.3: Mass loss during pupation (mg) =/-SE of male and female *C. eriphyle* for 2011 (A) and 2012 (B) Gunnison is represented by squares and Montrose Valley by circles.

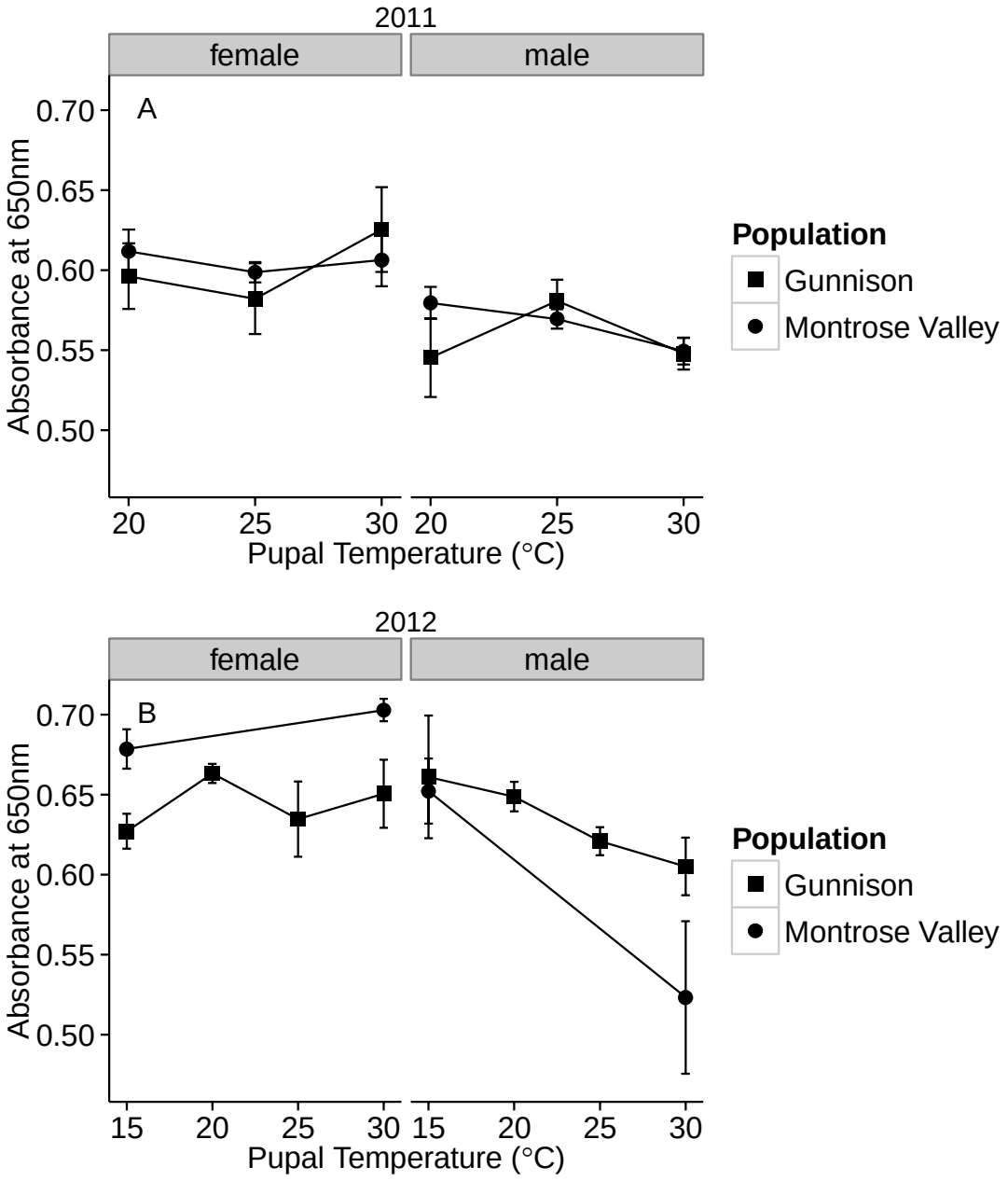

Figure 4.4: Wing absorbance at 650nm for males and females by year 2011 (A) and 2012 (B) Gunnison is represented by squares and Montrose Valley by circles.

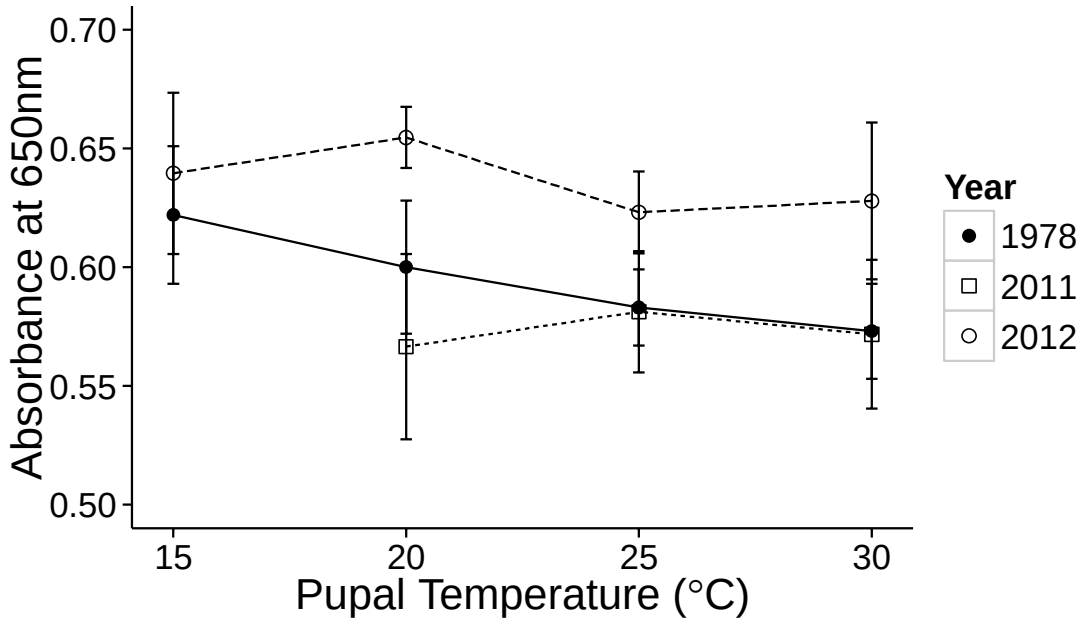

Figure 4.5: Wing absorbance at 650nm (+/-CI) comparing historical (1978, closed circle) and current (2011, open square), (2012, open circle).

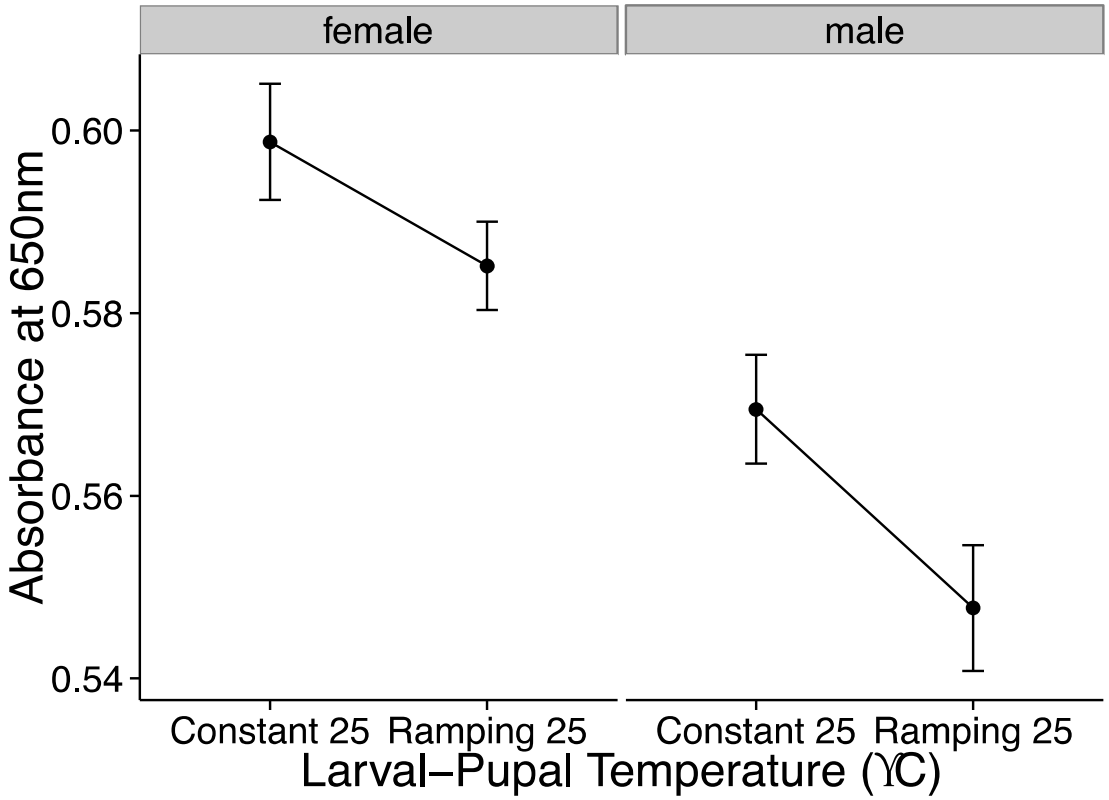

Figure 4.6: Wing absorbance at 650nm (+/-SE) for 2011 Montrose Valley animals reared either at a constant 25°C or an average 25°C (ramping 21-29°C).

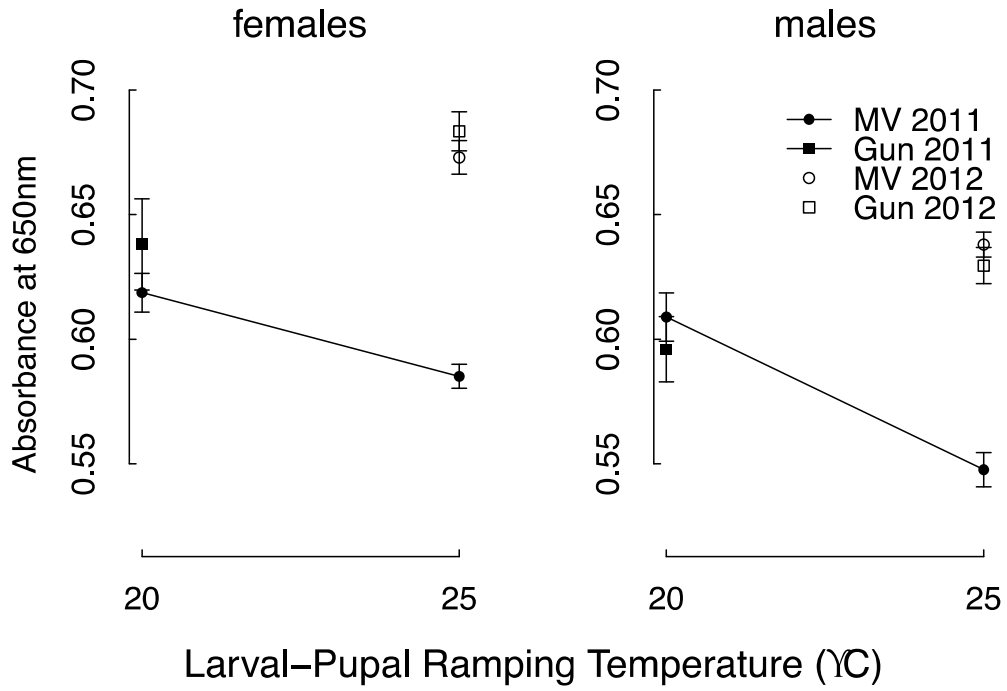

Figure 4.7: Wing absorbance at 650nm for females and males by year. Montrose Valley (circles) and Gunnison (squares). In 2011 Gunnison was only measured at 20°C and in 2012 both Montrose Valley and Gunnison were only measured at 25°C.

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CHAPTER 5: LOCAL ADAPTATION OF INSECT HERBIVORES TO HOST PLANTS DEPENDS ON TEMPERATURE

Introduction:

Many herbivorous insects are specialists that feed on a restricted set of host plant species within a single plant family. Across the geographic range of a single herbivore species, insect populations may become locally adapted to different host plant species (Fox & Morrow 1981; Dobler *et al.* 1996; Pelini *et al.* 2010). Local adaptation is driven by aspects of host plant quality including abundance, plant defenses, and plant nutrient levels (Fox, Waddell & Mousseau 1994; Jongsma & Bolter 1997; Egan & Ott 2007). Herbivore populations may utilize and adapt to novel host plants introduced into their range. Recent studies have demonstrated that evolutionary responses to novel host plants can occur quite rapidly, both for invasive plants (Harvey *et al.* 2010) and agricultural crops (Hare 1990; Gray *et al.* 2009).

Novel host plant species may be introduced or spread into only part of the geographic range of an herbivore species, such that different herbivore populations experience and adapt to different novel and native host plants. If climate and other environmental factors vary across the range, then host plant use and climate may co-vary among herbivore populations. Several recent studies with herbivorous insects have demonstrated that host plant and rearing temperature can interact to influence larval growth and development (Angilletta 2009; Pelini *et al.* 2009; Diamond & Kingsolver 2012; Clissold, Coggan & Simpson 2013). While rapid host plant shifts have been found

in other insects such as the soapberry bugs, which have adapted to feed on the fruits of introduced plants within the past 100 years (Carroll & Boyd 1992) the effects of environmental temperature on local adaptation to novel host plants have not been considered.

The sulphur butterfly *Colias eriphyle* occurs in open habitats in the western US, and at elevations of 1,400-2,900m in western Colorado. The larvae feed on plants in the *Fabaceace* family, particularly *Medicago sativa* (alfalfa), *Vicia* (vetch) *spp.,* and *Trifolium* (clover) *spp*. Agricultural alfalfa (*M. sativa*) was introduced to the Montrose Valley of Colorado during the early 1900s with the creation of the Gunnison Tunnel. This irrigation project diverted water from the Gunnison River into the Montrose Valley to enable agriculture (Page & Page 1907). Because of alfalfa's economic value, it has largely overtaken the native vetch species that was previously abundant. In regions at higher elevations in Colorado, such as the Gunnison Valley, native vetch remains a commonly occurring plant because of poor conditions for agriculture including dry soil, short growing season, and harsh winters, although some alfalfa can be found.

(Tabashnik 1983) studied *C. eriphyle's* response to the shift from native *Vicia* (vetch) and *Lathyrus* (sweet pea) to the introduced agricultural *M. sativa* (alfalfa). He used populations of *C. eriphyle* from Montrose Valley where *M. sativa* was present and from Gunnison where *M. sativa* was far less abundant. A common-garden study at a single rearing temperature suggested local adaptation to alfalfa in the Montrose Valley population, but not in the Gunnison population (Tabashnik 1982, 1983). In addition to host plant occurrence, the climatic conditions between the two sites are different and also changing due to climate change.

Climatic conditions differ between the Montrose (elevation 1.6km) and Gunnison (elevation 2.3km) regions: the growing season is shorter and ambient temperatures lower in Gunnison than in Montrose Valley (Higgins *et al.* 2013). Additionally, climate in western Colorado has changed rapidly with the frequency of warm days and nights increasing over the past 40 years (Booth, Byrne & Johnson 2012). Over the same period of time, *C. eriphyle* larvae from Montrose Valley have adapted to feed at higher temperatures that have increased in frequency (Higgins *et al.* 2013). However, little is known about whether there have been changes in host plant adaptation over this time period. In addition, we extend (Tabashnik 1983) study by examining how temperature can affect the pattern of local adaptation to host plant (Diamond & Kingsolver 2012).

In this study we measure survival, development time, and pupal mass of *C. eriphyle* larvae reared in the lab at two temperature regimes from both populations on native and introduced host plants. We predict to see evidence of local adaptation (high survival, shorter development time, larger pupal mass) of each population to its most abundant host plant. Additionally, we expect that the Montrose Valley population should have higher survival, shorter development time and larger pupal mass when reared under warmer conditions compared to the Gunnison population. We evaluated whether patterns of host plant adaptation have changed in these herbivore populations over the past 34 years (120-200 generations).

Methods

Colias eriphyle were collected from two sites in Colorado. The Montrose Valley, CO (N38.62, W108.02, 1,633 m) population was collected in agricultural alfalfa (*M. sativa)* fields. Its growing season is from April through October resulting in 3-5

overlapping generations of *C. eriphyle* per year (Tabashnik 1980), and the mean summer temperature is 22.5°C. The other population was collected from a county park in Gunnison, CO (N38.56, W106.94, 2,347 m) with vetch (*Vicia)* and clover (*Trifolium*) as the primary host plants. This population has a growing season of June through September resulting in two distinct generations per year (Watt, Han & Tabashnik 1979), with a mean summer temperature of 16°C. The current populations were selected to be within 5km of previous collection sites for the historical studies.

Adult female butterflies were shipped overnight to the laboratory at the University of North Carolina at Chapel Hill and kept in cages at greenhouse conditions (~26°C) under natural light. Females were fed 10% honey water solution by moistened sponge changed daily, and were allowed to oviposit on either potted vetch (*Vicia villosa)* or alfalfa (*M. sativa)*. Host plants were grown in the greenhouse from seeds (Johnny's Select Seeds, Waterville, Maine) and were approximately three-four weeks old before being used in the treatment. Plants were watered daily and fertilized weekly. Broods were split so that larvae from each family were reared on both host plants. Eggs were removed daily and placed in environmental chambers (Percival 36VL, Geneva Scientific, WI, USA) with diurnally fluctuating temperature regimes of either 16-24°C (average 20°C) or 21-29°C (average 25°C) and a 14L:10D photoperiod. The latter temperature regime was chosen to mimic the exact temperature protocol used by Tabashnik (1983). The temperature regimes fluctuated as a sawtooth with the high and the low separated by 12 hours. For logistical reasons, experiments at the two rearing temperatures were done at different times. For the larvae reared in the average 25°C conditions there were 375 total larvae from 15 different families from Gunnison and 15 families from Montrose Valley

with 2-37 (mean: 12.5) larvae from each family. For the larvae reared at the average of 20°C treatment there were 102 larvae total comprised of 7 families from Gunnison and 8 families from Montrose Valley with 1-16 larvae (mean: 5.7) from each family. Larvae were raised individually in petri dishes, and fed cut vetch or alfalfa leaves *ad. libitum.* Survival to pupation, time to each instar (following the 3rd instar), mass at each instar (following the 3rd instar), and mass 48 hours after pupation were recorded. Our analyses focused on survival to pupation, development time to pupation and pupal mass as response variables. All analyses were conducted using the R (3.0.2) statistical program with host plant and population as fixed effects and sib-family (mom) as a random effect. Survival data from 2012 was analyzed using binomial generalized linear mixed effects models using the *lme4* package (Bates *et al.* 2014) with population and host plant as fully crossed fixed effects and family as a random effect. Development time and pupal mass data were analyzed using linear mixed effects models using the *nlme* package (Pinheiro *et al.* 2014) with population and host plant as full crossed fixed effects and family as a random effect. Data for the two rearing temperatures were analyzed separately because the two experiments were conducted at different times.

Results

Survival

Mean survival to pupation was highest for each population on its local host plant at both temperatures. The larvae from Montrose Valley had highest survival on alfalfa, whereas the larvae from Gunnison had highest survival on vetch at both temperatures (Figure 5.1). Both population ($z= 2.68$, $p<0.01$) and the interaction between host plant

and population (z=-4.41, p<0.001) were significant at 20°C. At 25°C there was no statistical difference in survival among host plants or populations.

Days to Pupation

Both population ($F_{1,8}$=175.5, p<0.0001) and host plant ($F_{1,90}$=37.37, p<0.0001) significantly affected development time to pupation at 20°C, but there was no significant interaction between the two ($F_{1,90}$=2.07, p=0.15). At 20°C, mean development time was 10-15 days longer for the Gunnison than the Montrose Valley population on both host plants, and was longer on vetch than alfalfa for both populations (Fig 5.2). At 25°C, mean development times differed by less than a day for the two populations and host plants (Fig 5.2), and there was no significant effect of population ($F_{1,19}$=0.08, p<0.78), host plant ($F_{1,352}$=0.08, p<0.51), or their interaction ($F_{1,352}$=3.14, p<0.08).

Pupal mass

At 20°C pupal mass was significantly affected by population ($F_{1,8}$=22.46, p<0.002) with the larvae from Gunnison taking longer to develop and by the interaction of population and host plant ($F_{1,87}$=4.20, p=0.04), but not by host plant alone ($F_{1,87}$=0.04, p=0.83). At 20°C the pupae from the Montrose Valley were larger than those from Gunnison, with a larger difference on vetch than on alfalfa (Fig 5.3). There was no significant effect of population ($F_{1,19}$=0.03, p=0.87), or host plant ($F_{1,348}$=3.2, p=0.08), at 25°C, but there was a significant interaction of population and host plant ($F_{1,348}$=36.67, p<0.0001). At both rearing temperatures the interaction is in the opposite direction predicted by local adaptation: mean pupal mass was greater on vetch than on alfalfa for the Montrose Valley population, and larger on alfalfa than on vetch for the Gunnison population (Fig 5.3).

Comparison to historical data

Based on a common-garden study at 25°C (see Methods), Tabashnik (1983) found larvae from Gunnison took longer to develop on alfalfa than did the larvae from Montrose Valley (Fig 5.4A), however this effect was not seen in 2012 (Fig 5.4B). The historical study found no effect of population on pupation mass, whereas in 2012 there was a host plant and population interaction (Fig 5.4B). Pupal masses were larger in the 1978 than in the 2012 study on both host plants.

Discussion

We examined the interactions between climate, specifically temperature, and host plant adaptation in two populations of *Colias* larvae that differ in the length of the growing season and annual number of generations. Tabashnik (1983) demonstrated that larvae in Montrose Valley had adapted to introduced alfalfa by showing a decreased development time when larvae were fed alfalfa. Our results suggest that this adaptation is still occurring, however it is occurring only in regards to survivorship.

Host plant shifting to an introduced species is common for herbivores. Some of the effects of host plant shifting can be dramatic such as the hybridization of two *Rhagoletis* species following the introduction of the *Lonicera* (honeysuckle) plant (Schwarz *et al.* 2005). Additionally, the rapid radiation of Lepidoptera species is thought to be product of host plant expansion (Fordyce 2010). Host plant expansion can also have less drastic results. After the introduction of *Plantago lanceolata,* the *Euphydryas editha* butterfly began using it as a suitable host plant. The *E. editha* began to show oviposition preference for the introduced plant even though there was no difference in other fitness components when larvae were fed either *P. lanceolata* or the original host plant,

Collinsia parviflora (Thomas *et al.* 1987). This suggests that host plant shifting and expansion can begin gradually, and only affect specific fitness components. What we have shown in our study is that the effects of host plant adaptation are dynamic over time.

We found that the thermal environment can alter differences in larval performance on host plants. When the larvae were reared at 25°C there was no difference in development time between the populations. However, when the larvae were reared at 20°C the development time differences between populations and host plants were far more exaggerated. At 20°C the larvae from both populations had longer development times, especially the larvae from Gunnison that took 10-15 days longer to develop. The Montrose Valley pupae were larger despite the shorter development time. Paradoxically, at 20°C, the pupal masses were highest on the less abundant host plant for each population. The Gunnison pupae were largest on alfalfa and the Montrose Valley pupae were largest on vetch, despite development time being shorter on alfalfa for both populations.

The lower temperatures used in our experiment were within the normal range of temperatures experienced by *Colias* in the field, but they did enhance the effects of the different host plants (i.e. longer development time on vetch) for each population. During their respective growing seasons, larvae in Montrose Valley spend slightly less time at 20°C than do larvae in Gunnison. The larvae from Montrose Valley eat at about the same rate as those in Gunnison at 20°C (Higgins *et al.* 2013). However, these results are from short term (30 minute) feeding bouts so the long term effects of exposure to 20°C may not be evident. Tabashnik (1982) looked at mean mass and growth rate of 3rd instar *C. eriphyle* from Gunnison and Montrose Valley reared on vetch and alfalfa at 18°C (high

22°C; low 14°C). The larvae from both populations had larger masses on alfalfa as compared to vetch, but this omits the 5th instar when *Colias* larvae gain more mass than in any other instar. These data contrast with the present study, where larvae from Montrose Valley had larger pupal masses when fed on vetch at 20°C. Responses by other insects to host plants have been shown to vary with environmental temperatures. In translocation experiments using *Papilio zelicaon,* larvae from both core and periphery populations had higher survival and pupal mass when reared at the periphery (cooler) thermal conditions and showed differing host plant preferences for each rearing condition (Pelini *et al.* 2009).

We know that climate has changed in the past 40 years in these sites particularly with increases in temperature variability (Higgins et al. 2013), however our final question was to see if larval performance and local adaptation to host plants has changed over the same amount of time. In Tabashnik's (1983) study, both populations had higher survival and larger pupal mass on alfalfa than on vetch. His clearest evidence for local adaptation was that development time was much longer on alfalfa than on vetch for the Gunnison population, but not for the Montrose Valley population (Fig 5.4A). In the present study, development times were similar for both populations on both host plants (at 25°C). However, it is worth noting that in both years development time that it was slightly (albeit not significantly) longer on alfalfa compared to on vetch in Montrose Valley (Fig 5.4A).

Our survival data shows the local adaptation pattern that was expected: each population has the highest survival rate on the host plant that is most abundant. This pattern of local adaptation is particularly striking at the lower rearing temperature.

Both studies found differences in pupal mass. In the historic study, the larvae from Gunnison that took about 4 extra days to develop on alfalfa versus on vetch were approximately 10-15 mg larger as pupae. In the current study, Gunnison larvae that consumed alfalfa were ~15-20 mg larger as pupae than the larvae that consumed vetch despite similar development times. The pattern of the Gunnison population having larger pupal masses on alfalfa versus vetch was evident in both years (Fig 5.4B). The relative difference of pupal size in the two studies suggests that alfalfa may be a more nutritious host plant despite it not being common in Gunnison. Additionally, Tabashnik (1982) reported that relative growth rates were significantly higher for both populations when they fed on alfalfa. In agricultural studies, alfalfa typically has a higher nitrogen concentration than vetch, but the difference is variable (Brady 1982, Badaruddin and Meyer 1990). Tabashnik (1983) found that females from both populations showed an oviposition preference for alfalfa, which may signal that the adults can recognize alfalfa as a more nutritious host plant than vetch. Additionally, we do not see evidence that the Montrose Valley population is losing its ability to consume vetch as it has a slightly higher pupal mass on vetch compared to alfalfa, which was reported by Tabashnik (1983). It is also possible that the different host plants cause for tradeoffs in life history parameters.

In Tabashnik's (1983) study the vetch used was cut from the field and alfalfa was grown in pots versus in our study where both host plants were grown in pots and fertilized weekly. These non-optimal rearing conditions may have selected for faster growing and bigger larvae more likely to survive, although we do not know about survival in the previous study. In the present study, survival was relatively high overall

for both host plants (Fig 5.1). The difference in rearing conditions could explain some of the differences seen in pupal size and development time between the two studies. In addition to rearing differences there may also be genetic differences between the plants used in our study compared to the historical study, however the relative comparison of each population on both host plants is still a worthwhile comparison.

Our evidence of local adaptation to host plant is restricted to larval survival to pupation. This suggests that the mechanisms of adaptation (survival, development time, pupal mass) are dynamic over time. The changing of adaptation mechanisms including the loss of adaptation to one or another fitness metric is a unique finding and an interesting area for further study and research.

FIGURES

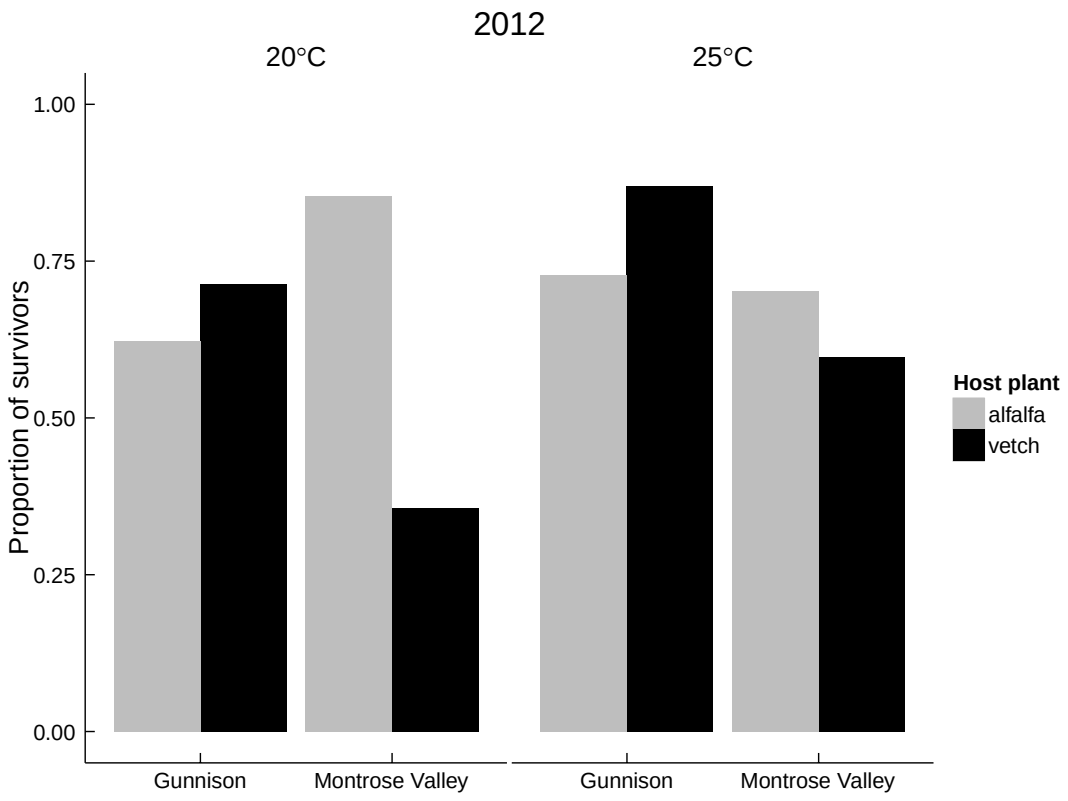

Figure 5.1: *C. eriphyle* survival at 20 and 25°C in 2012 from Gunnison and Montrose Valley on alfalfa and vetch.

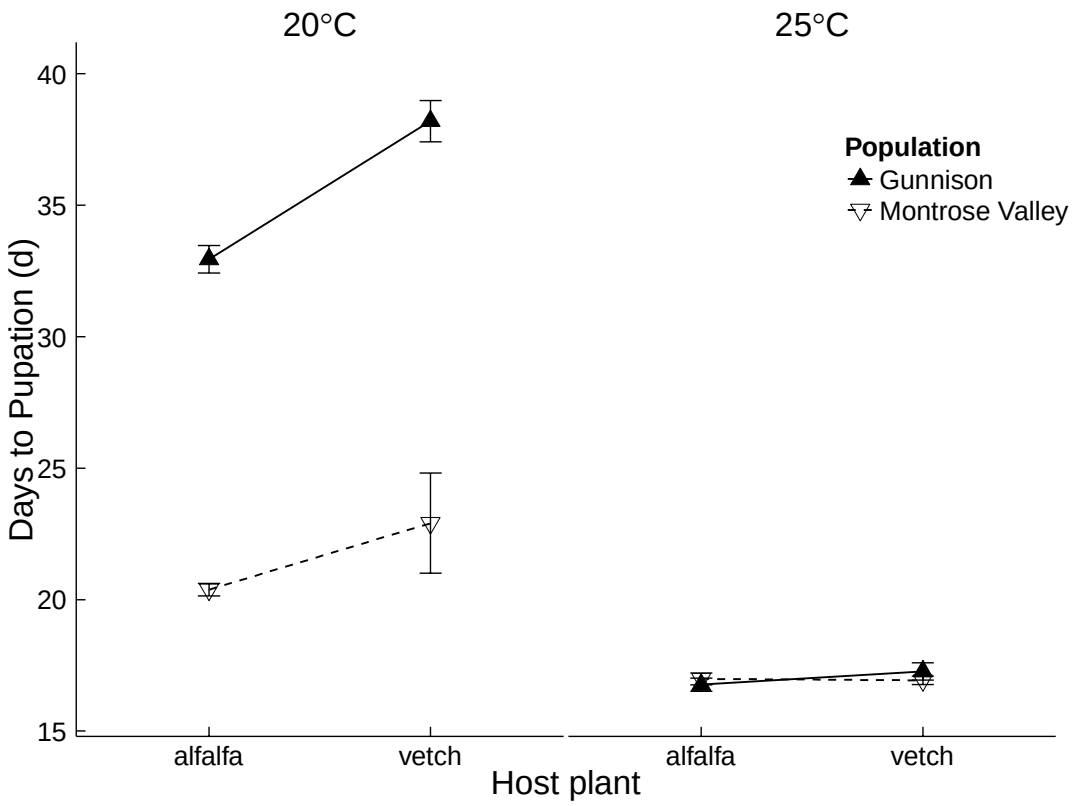

Figure 5.2: *C. eriphyle* days to pupation (+/- SE) on each host plant at 20°C and 25°C for Gunnison (solid line, up filled triangles) and Montrose Valley (dotted line, down open triangles).

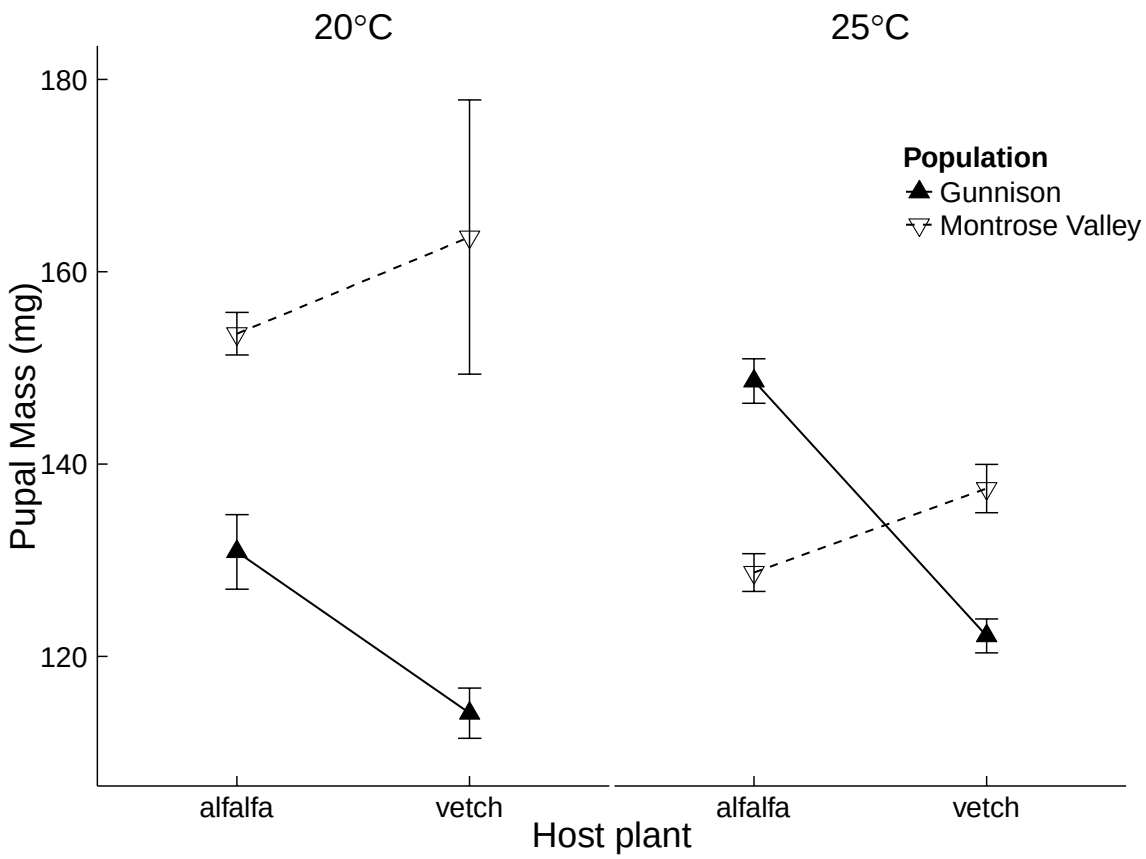

Figure 5.3: *C. eriphyle* pupal mass (+/- SE) on each host plant at 20°C and 25°C for Gunnison (solid line, up filled triangles) and Montrose Valley (dotted line, down open triangles).

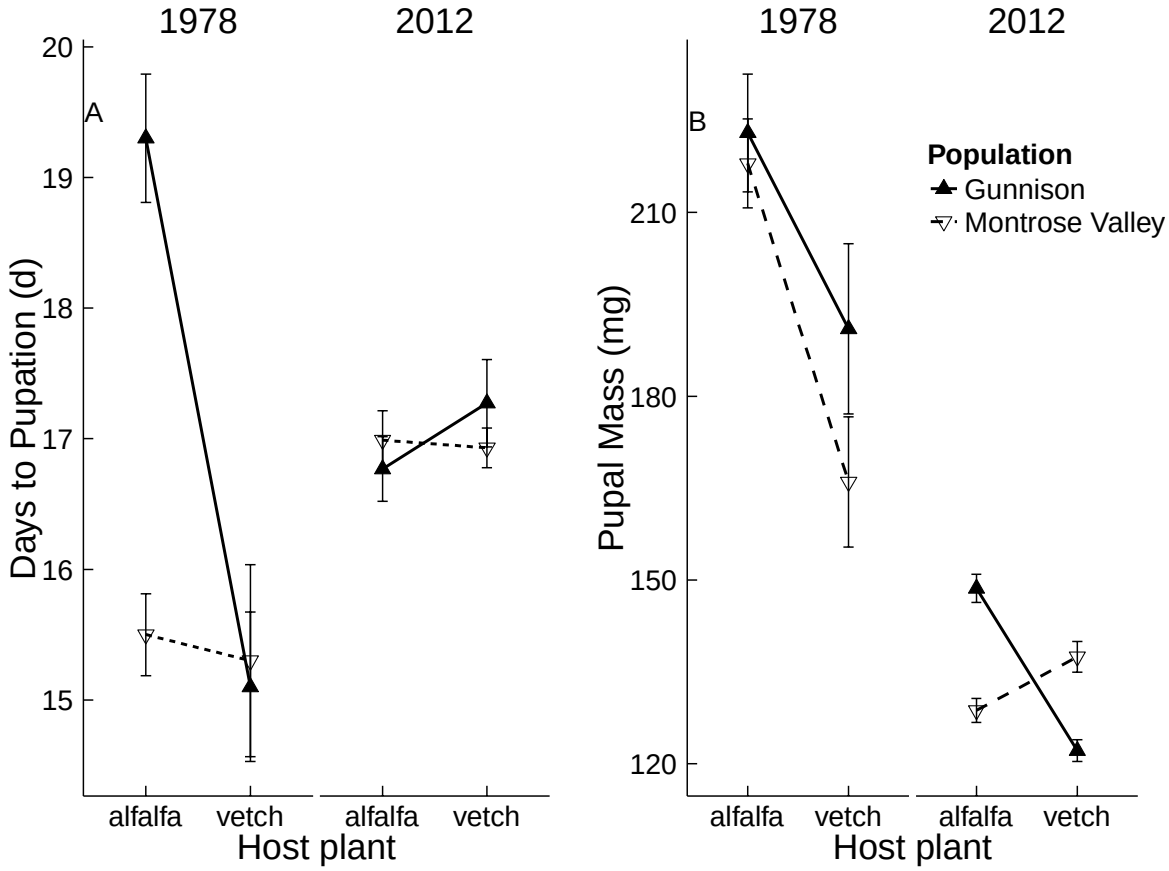

Figure 5.4: *C. eriphyle* development time (+/- SE) (A) and pupal mass (+/- SE) (B) and for larvae reared at 25°C in 1978 and 2012 for Gunnison (solid line, filled up triangles) and Montrose Valley (dashed line, open down triangles).

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