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Geographic differences and microevolutionary changes in thermal sensitivity of butterfly larvae in response to climate

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Summary

1. Rapid evolution of physiological traits in response to novel thermal environments has rarely been demonstrated in natural populations.

2. We studied the temperature dependence [thermal performance curves (TPCs)] of larval feeding rate for two populations each of *Colias eurytheme* and *Colias eriphyle* in North America that occur over a range of elevations and climates. Using historical data for two of the populations, we assessed changes over time in both air temperatures and TPCs for larval feeding.

3. Populations at lower elevations with longer growing seasons had broader TPCs for larval feeding. In contrast, higher elevation populations with shorter growing seasons had higher optimal and maximal temperatures for feeding.

4. Overall mean air temperatures during the growing season showed little change at the two sites, but the frequency of high air temperatures (> 28 °C) has increased markedly at both sites over the past 40 years. This climatic shift was associated with increased rates of larval feeding at higher temperatures (> 28 °C) in both populations.

5. These results suggest that recent climate warming has led to physiological shifts in the TPCs for larval feeding in this system, indicating that thermal adaptation can occur rapidly in response to changing thermal conditions.

Key-words: adaptation, climate change, *Colias*, thermal performance curve

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, behaviour (Dingle, Mousseau & Scott 1990), thermal performance and thermal tolerance (Damme *et al.* 1989; Stevens 1992; Gaston & Chown 1999; Badyaev & Ghalambor 2001).

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 90th percentile recorded from 1961 to 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 (Walther *et al.* 2002; Parmesan & Yohe 2003). 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 (Réale *et al.* 2003), mosquitoes (Bradshaw & 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 & Holzapfel 2007; Karell *et al.* 2011). To date, evidence for evolutionary responses in

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thermal physiology to recent climate change has been limited (Huey, Patridge & Fowler 1991; Stillman 2003). 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 *Colias eriphyle* and closely related species demonstrate morphological adaptation to temperature in wing melanism and thorax fur thickness (Watt 1968; Kingsolver 1983; Kingsolver & Watt 1983). Little is known about *Colias* larvae and if they also display local adaptation to climate (Sherman & Watt 1973). Rates of larval feeding, growth and development are essential to success and are strongly temperature-dependent in most insects (Stamp & Casey 1993). The primary function of the larval life stage is to assimilate nutrients, and larvae do this by near-constant feeding. Sherman & Watt (1973) measured short-term rates of larval feeding in two *Colias* species: *Colias eurytheme* from the Sacramento Valley in California (19 m) and *C. eriphyle* from the Montrose Valley in Colorado (1633 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 remeasuring 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 & 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 & Watt 2008). The larvae for both species have five larval instars and *C. eriphyle* undergo a facultative diapause during the third instar, whereas *C. eurytheme* overwinter as quiescent larvae. The larvae for both species feed on plants in the *Fabaceace* family, particularly *Medicago 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 2000 m. *Colias eriphyle* occurs in open habitats in the western US, and in western Colorado, it is found at elevations of 1400–2900 m.

We collected *Colias* from four sites for these studies. To allow historical comparisons with Sherman & Watt (1973), we sampled *C. eriphyle* females from alfalfa (*M. sativa*) fields located in the Montrose Valley, CO (N38·62, W108·02, 1633 m); and *C. eurytheme* females from alfalfa fields in the Sacramento Valley, CA (N38·44, W121·86, 19 m). To expand the geographic 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, 2347 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 *Colias 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 eight to nine generations of *C. eurytheme* per year. In Chapel Hill, NC, the season starts in March and ends in November resulting in three to five 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 three to five 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 (*c.* 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, Fontana, WI, USA) maintained at 25 °C on a 14 L/10 D photoperiod where larvae were given leaves of *V. villosa ad libitum*. Upon entering the fifth instar, larvae were starved for 3 h and weighed. The larvae were then exposed to one of 5–10 different experimental temperatures between 15 and 35 °C and allowed to acclimate for 15 min before cut *V. villosa* leaves were added. Once the *V. villosa* was added, the larvae were allowed to feed for 30 min. To ensure experimental temperatures above the optimal temperature for feeding were included, some populations were measured at several additional temperatures between 38 and 43 °C.

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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 & Watt (1973) study. First, Sherman and Watt quantified feeding rate ($mm^2\ s^{-1}$) 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 min trial, thus mass gained directly reflects consumption. Secondly, Sherman & 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 two to four 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 to 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 analysed using the R (15.1, R Core Team, Vienna, Austria) statistical package. Feeding rate was defined as $F = \frac{\ln(\text{Final Mass}/\text{Initial Mass})}{\text{Time Spent Feeding}}$. This represents the proportional rate of mass gain of a larva. Feeding rates were analysed 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, $\sigma = 0{\cdot}012$; individual within family, $\sigma = 0{\cdot}015$). 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 & Berrigan (2006), which is the product of a Gaussian function and a Gompertz function:

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

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 & Stevenson 1993; Bauwens *et al.* 1995).

Results

DIFFERENCES IN THERMAL PERFORMANCE CURVES AMONG CURRENT POPULATIONS

Thermal performance curves for feeding rate differed substantially and significantly among populations (Fig. 1, Table 1). There were significant first- and second-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. 1).

These differences in TPCs can be characterized in terms of the key parameters (Frazier, Huey & Berrigan 2006) that describe TPCs (Table 2). Comparing all four of the

Table 1. Results of ANOVA for the effects of temperature (T) and population (P) on feeding rate in *Colias eriphyle* and *Colias 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. Bold indicates significance.

Parameter	d.f.	F-value	P-value
T	1	53·14	**< 0·0001**
T^2	1	130·16	**< 0·0001**
T^3	1	0·26	0·61
P	3	9·39	**0·0001**
$T{:}P$	3	15·19	**< 0·0001**
$T^2{:}P$	3	12·61	**< 0·0001**
$T^3{:}P$	3	1·60	0·19

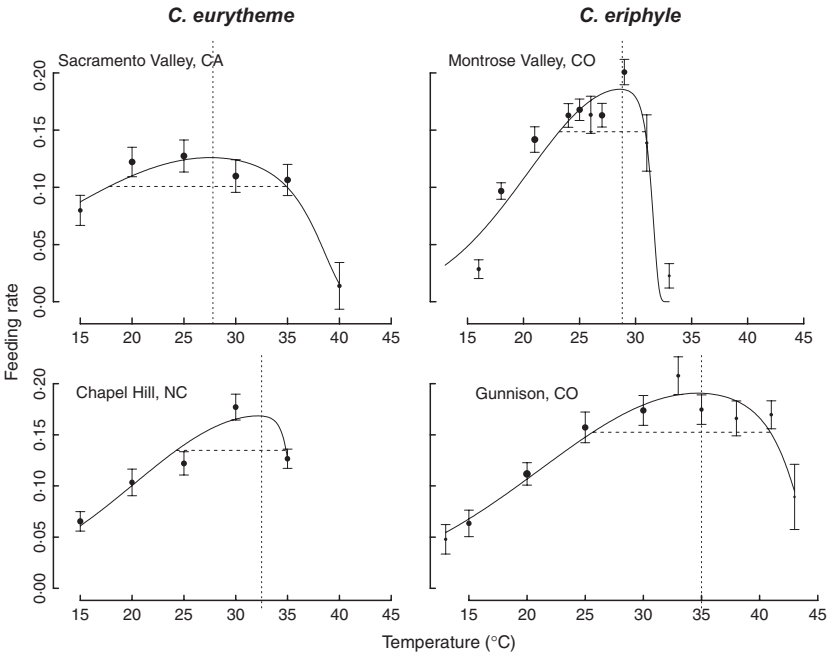

Fig. 1. The thermal performance curves (TPCs) for feeding rate [mean ± SE of ln (final mass/initial mass)/time] between the four populations. See the methods for an explanation of how changes in mass reflect short-term feeding rate versus growth. The curve is the fit of the (Frazier, Huey & Berrigan 2006) TPCs 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.

Table 2. Parameter estimates for the thermal performance curve (±SE)

Species	Population	F_{max}	T_{opt}	ρ	σ	B_{80}
Colias eurytheme	Sacramento Valley, CA – 19 m	0·13 ± 0·01	27·8 ± 2·1	0·54 ± 0·12	0·002 ± 0·001	17·1
C. eurytheme	Chapel Hill, NC – 148 m	0·17 ± 0·01	32·5*	1·87 ± 0·22	0·003 ± 0·0008	10·6
Colias eriphyle	Montrose Valley, CO – 1633 m	0·19 ± 0·02	28·8 ± 2·8	2·14 ± 3·10	0·007 ± 0·004	7·7
C. eriphyle	Gunnison, CO – 2347 m	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*

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}. Italics indicate historical data.

*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 s^{-1}, whereas for the 2012 experiment is was ln(final larvae mass/initial larvae mass)/time.

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 laboratory that exceed the climatic temperatures (T_{air}) they would normally experience in the field during their growing seasons (Fig. 2). The *C. eurytheme* populations experience longer growing seasons (365 and 275 days for Sacramento Valley and Chapel Hill populations, respectively) than the *C. eriphyle* populations (214 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, 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

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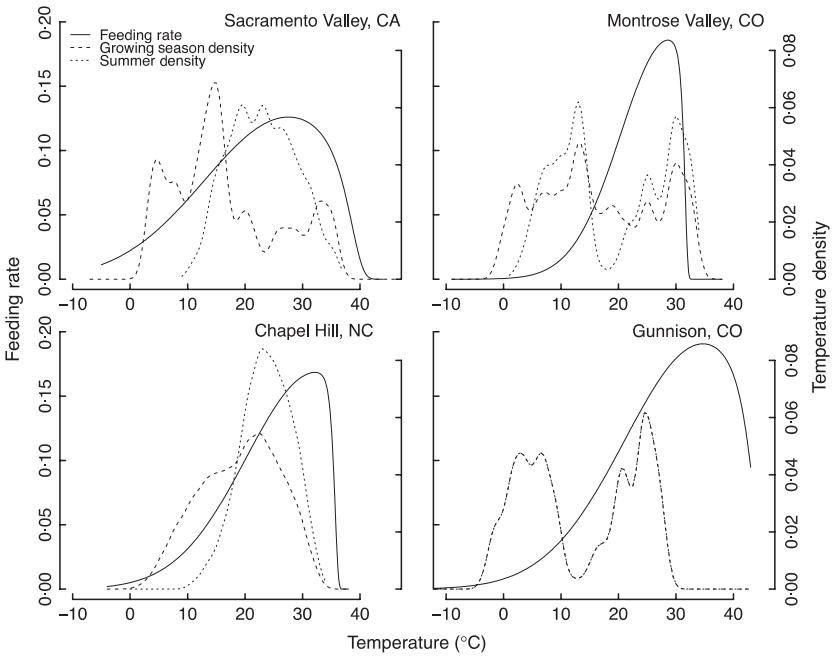

Fig. 2. Feeding rate (solid line) and T_{air} during the growing season for each population. The temperature density is depicted for both the appropriate growing season (dashed) and the summer months (June 1–September 30, dotted). The growing season and summer months are the same for Gunnison, CO.

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); 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 & Watt (1973) with our current results. TPCs 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. 3, Table 3). The significant first- and second-order temperature terms show differences in unimodal curvature. The interactions between the second-order temperature terms and year indicate significant differences in TPCs between the previous and current data (Table 3). Both populations have increased their capacity to feed at higher temperatures during the past 40 years (Fig. 3). In addition, during the past 40 years, T_{opt} increased by *c.* 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). 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. 3). While overall mean temperatures during the growing seasons show slight increase from the 1960s to the 2000s (18–19·5 °C in CA, and 13·5–14·5 °C in CO), 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 4·4–20% at Montrose Valley. As a result, climate change has increased the frequency of exposure to high air temperatures by two- to fourfold at these sites (Fig. 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 & 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.

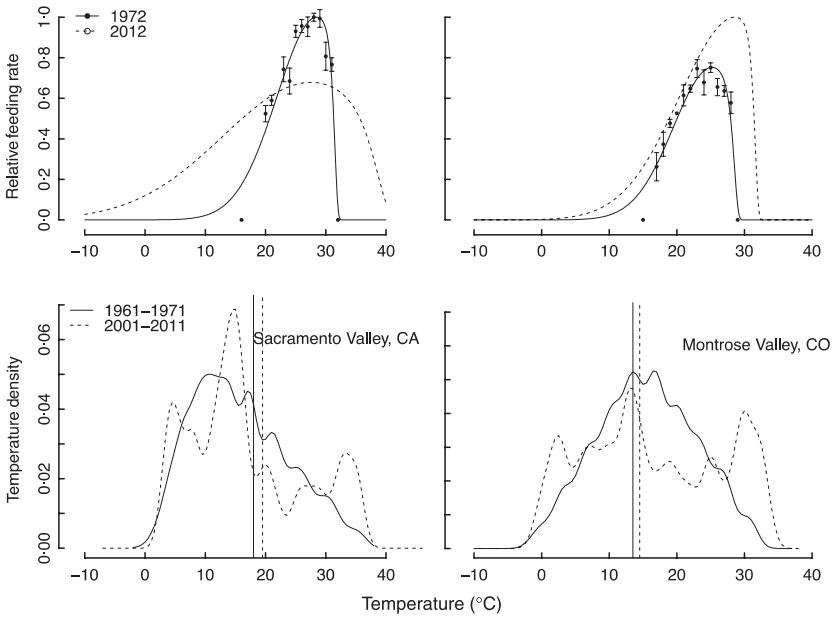

Fig. 3. Historical comparison of larval feeding rate and temperature density during the growth season for *Colias eriphyle* and *Colias eurytheme*. The solid line designates data from the past, and the dashed line is current data. The points are measured feeding rates in the Sherman & Watt (1973) experiment (mean ± SE). Relative feeding rate is calculated by standardizing the highest feeding rate for each year to one. The vertical lines indicate mean temperature.

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 feed 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 with the *C. eurytheme*. In addition, the T_{opt} was nearly as high as T_{air} for 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 & 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 h) maximal growth rates at 35 °C despite optimal long-term growth occurring at 30·5 °C (Kingsolver 2000).

Table 3. Results of ANOVA for the effects of temperature (*T*), population (*P*) and year (*Y*) in the historical comparison between 1972 and 2012 of *Colias eriphyle* and *Colias eurytheme* feeding rates. Bold indicates significance

Parameter	d.f.	*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

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 neotropical 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 & Gotelli 1999; Van Doorslaer & Stoks 2005). For example, Conover & 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 1983).

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 show more variability. There has been an increase in the density of higher temperatures (above 28 °C) from 2001 to 2011 versus from 1961 to 1971. The frequency of air temperatures above 28 °C has increased twofold in Sacramento Valley, CA, and more than fourfold 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, Barsugli & Averyt 2008).

The temperatures recorded at each weather station are the maximum and minimum T_{air} for the day measured 2 m 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 & 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 min 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 and 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 (Skelly *et al.* 2007; Visser 2008; Hoffmann & Sgrò 2011). While previous work has highlighted adaptation to seasonal timing, specifically photoperiodic cues (Bradshaw & 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.

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