Thermal Resistance and Performance Correlate with Climate in Populations of a Widespread Mosquito

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ABSTRACT

The abundance and success of widely distributed species across variable environments make them suitable models for exploring which traits will be important for resilience to climate change. Using a widespread mosquito species, *Culex tarsalis*, we have investigated population-level variation in the critical thermal maximum (CT_{max}) and the metabolic response to temperature (MR-T). Adult female *C. tarsalis* were sampled from three sites representing thermally distinct habitats in California, and flow-through respirometry was used to determine CT_{max} and MR-T relationships. CT_{max} differed significantly among the three populations and correlated positively with maximum temperatures at each site but not with mean temperatures. *Culex tarsalis* from our cool-temperature, high-altitude site had significantly higher metabolic rates at each test temperature compared with the two populations from warmer sites, consistent with previous examples of thermal compensation in ectothermic animals inhabiting cold climates. The MR-T slope was steepest in mosquitoes inhabiting the site with the lowest temperature variability, while shallower slopes were exhibited by mosquitoes from the two sites with higher thermal variability. Our results show the extent to which local populations may differentiate within their respective environments and suggest that plasticity in thermal tolerance traits may play a role in mediating resilience to climate change. Furthermore, our study highlights the importance of thermal variability and extremes rather than average temperatures for the evolution of thermal traits.

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Introduction

Physiological resistance to stressful temperatures is an important component of the fitness of any organism, particularly ectothermic species. Because of the effects of temperature on metabolic rate, geographic distribution, and survival in animals, responses to temperature have been a frequent focus of study in physiological ecology. As our knowledge of thermal performance and resistance in ectotherms develops, biologists have begun to explore how these traits contribute to the responses species will show to global climate change. Recent projections from the Intergovernmental Panel on Climate Change indicate that mean global temperature may warm as much as 6°C by the end of the twenty-first century (IPCC 2007). These rapid environmental shifts may induce a variety of responses in organisms, including migration (Parmesan 2006), shifts in phenology (Bradshaw and Holzapfel 2001), or changes in thermal tolerance traits (Dillon et al. 2010). These coping strategies will vary widely across species depending on dispersal ability, the degree of phenotypic plasticity, and the availability of adequate genetic variation for selection. Identifying these responses and the mechanisms underlying them is currently a major challenge in ecological research (Angilletta 2009).

Although many studies have sought to identify and predict climate change responses, this topic remains poorly understood. For example, two recent widely cited articles have established contradictory hypotheses about which species are most threatened by climate warming. Deutsch et al. (2008) assert that tropical species are most at risk because they currently live at temperatures much closer to their critical thermal maxima (CT_{max}) than temperate species (Deutsch et al. 2008; Tewksbury et al. 2008). On the other hand, Hoffmann (2010) argues that although the average temperatures at tropical sites are higher, temperate habitats experience both more drastic and more frequent bouts of extreme temperature than tropical habitats. The author concludes that because CT_{max}'s are similar in both high- and low-latitude species, tropical species are less likely to be exposed to lethal temperatures and are less threatened by climate change than temperate species (Hoffmann 2010).

These contradictory hypotheses clearly illustrate that large-scale, climate-based studies are not currently able to predict the biological effects of climate change. By contrast, studies of thermal responses in individual species can provide insight into the mechanisms underlying these responses. For example, experiments with *Drosophila* have shown that phenotypic plasticity rather than genetic differentiation may account for population-level differences in thermal tolerance traits (Ayrinhac et al. 2004; Hoffmann et al. 2005). Similar results have been reported in studies of other insects such as the tsetse fly *Glossina*

Table 1: Climatic characteristics of *Culex tarsalis* collection sites

Location (coordinates [°]; altitude [m]) and collection dates	Mean temperature (°C)	Mean maximum temperature (°C)	Highest temperature (°C)
Coachella (33.53, −116.10; −70)	21.4	30.2	
March 25, 2011			24.4
April 25, 2011			36.1
October 22, 2011			39.4
Irvine (33.67, −117.84; 3)	20.8	24.2	
July 1, 2011			29.4
July 6, 2011			30.6
July 25, 2011			27.8
Mammoth Lakes (37.61, −118.82; 2,149)	16.1	27.2	
July 12, 2011			30.6
July 17, 2011			26.7
July 23, 2011			28.9

Note. Mean and mean maximum temperatures, respectively, represent the mean temperature and the mean daily maximum temperature for the month(s) collection took place. Highest temperature represents the highest value recorded during the week before each collection date.

pallidipes (Terblanche et al. 2006). By contrast, evidence for genetic differentiation in thermal tolerance traits has been reported in intertidal whelks *Nucella canaliculata* (Kuo and Sanford 2009; Somero 2010) and the mosquito *Anopheles gambiae* (Gray et al. 2009; Rocca et al. 2009). A series of investigations on the pitcher plant mosquito *Wyeomyia smithii* have revealed a complex interplay between seasonal photoperiod and ambient temperature in setting the thermal tolerance parameters of this temperate species (Bradshaw et al. 2004; Bradshaw and Holzapfel 2008; Ragland and Kingsolver 2008). While these and other studies have provided valuable insight into mechanisms underlying thermal trait variation, they may also contribute insight into which factors are likely to determine the "winners" and "losers" in climate change (Somero 2010). We suggest that species with broad geographic distributions have been underinvestigated in this context and that much insight can be gained from studying such "winners."

In this study, we have investigated thermal tolerance and performance in the widespread mosquito species *Culex tarsalis*. *Culex tarsalis* is distributed throughout the western half of North America, where it thrives over a wide range of latitudes and altitudes that encompass the scope of thermal extremes found in the continental United States (Darsie and Ward 2005). Furthermore, a population genetic study of this species has revealed the patterns of gene flow among populations, indicating the existence of three population clusters that are genetically isolated from one another (Venkatesan and Rasgon 2010). The broad distribution and genetic information available for this species make *C. tarsalis* particularly amenable to better understanding the roles of various physiological traits in producing "winners." *Culex tarsalis* is also medically significant because of its role as the principal vector of St. Louis encephalitis, western equine encephalitis, and West Nile virus in the western United States (Reisen et al. 2008). Therefore, investigating the thermal responses of this species is also invaluable for predicting the future distribution of important human and animal diseases.

The specific aim of this study is to determine whether populations of *C. tarsalis* inhabiting different climatic regions exhibit physiological clines in two thermal response traits. We compared CT_{max} and metabolic responses to a range of temperatures (MR-T) among three populations of *C. tarsalis* mosquitoes collected from a desert region, a coastal region, and a subalpine region. We predicted that populations would exhibit thermal physiologies specific to the environment in which they live.

Material and Methods

Field Sampling and Study Animals

Adult female *Culex tarsalis* mosquitoes were collected from three sites representing distinct thermal habitats within California (table 1). Recent studies have revealed the existence of three genetically distinct population clusters of *C. tarsalis* in the western United States (Venkatesan and Rasgon 2010). According to these data, our sites include two genetically distinct populations (Coachella, Mammoth Lakes) and one intermixed population (Irvine). All mosquitoes were sampled during the seasons of peak abundance at each site (July in Mammoth Lakes and Irvine; April and October in Coachella).

Adult blood-seeking females were collected using carbon dioxide–baited traps set out overnight at each sampling site and transported the following morning to the nearest laboratory facility (University of California, Irvine, or the Sierra Nevada Aquatic Research Lab, Mammoth Lakes, CA) for experiments. Collected mosquitoes were identified using keys based on adult morphological characters (Darsie and Ward 2005), and *C. tarsalis* were isolated and housed in 30 × 30 × 30-cm insect cages labeled with the date and location of capture. Cages were kept in a temperature-controlled room at 25°C and maintained on

a 12L : 12D cycle. Mosquitoes were provided daily with cotton balls soaked in 10% sucrose, and damp paper towels were placed over each cage to increase relative humidity. Experiments were performed with all Irvine and Mammoth Lakes mosquitoes within 9 d of capture. Because of constraints on collection at our Coachella site, experiments were performed within 20 d of capture for Coachella mosquitoes. Before all experiments, mosquitoes were fasted overnight by removing them from the insect cages and placing them into a small holding chamber where they were provided with deionized water from a soaked cotton ball.

CT_{max}

Thermolimit respirometry (Lighton and Turner 2004) was employed using a flow-through system to estimate CT_{max} in mosquitoes from each collection site (Coachella $N = 25$; Irvine $N = 15$; Mammoth $N = 23$). A programmable temperature-controlled cabinet (Sable Systems International, Las Vegas, NV) controlled by a Peltier device (PELT-5, Sable Systems International) was used to manipulate the temperature of air inside the 2-mL chamber containing the mosquito. At the start of each trial, a baseline recording was taken for 2 min while the respiratory chamber was empty. The recording was paused while a mosquito was placed inside the chamber and was resumed after 2 min. At this point, the temperature profile programmed into the PELT-5 was initiated, starting with a 20-min equilibration period at 25°C followed by heating at 0.5°C min^{-1} up to 60°C. This heating rate was selected as the fastest rate that still allowed thermal equilibration between the mosquito and the surrounding air, thereby avoiding lag effects (Lighton and Turner 2004). Faster rates were desirable for these very small insects to minimize exposure to dry air and prevent desiccation stress. The temperature of the air flowing past the mosquito was measured simultaneously with CO_2 output during each trial using a thermocouple inserted into the tubing immediately downstream from the insect and recorded using a thermocouple meter (TC-2000, Sable Systems International).

Flow-through respirometry was conducted by pumping air through three scrubber columns: two with silica gel to remove water vapor and one with Ascarite/Drierite to remove CO_2 and any remaining water vapor. The scrubbed air passed through a mass-flow controller (0–200-mL model, Side-trak, Sierra Instruments, Monterey, CA) that regulated air flow at 100 mL min^{-1} before the airstream entered the temperature-controlled cabinet. Inside the cabinet, a coil of copper tubing (160 cm long) was used to ensure thermal equilibration of the air before it entered the 2-mL respiratory chamber containing the mosquito. The air leaving the respiratory chamber was then directed out of the temperature-controlled cabinet and into an infrared gas analyzer (LiCor, model LI-6251, Lincoln, NE) where the CO_2 emitted by the mosquito was measured. Infrared activity detection was also used in a subset of samples ($N = 5$) to verify that the cessation of motor activity and spiracular activity coincided. Two infrared diodes (AD2 Infrared Activity Detector, Sable Systems International) were placed on either side of the respiratory chamber and anchored using modeling clay to point directly at the insect. The analog outputs from the LiCor, AD2, and TC-2000 were directed to a laptop computer via a data acquisition interface (UI2 Interface, Sable Systems International). CO_2 concentration (ppm), activity (arbitrary units), and temperature of the airstream (°C) were recorded every 1 s for the duration of each trial using Expedata software (Expedata PRO, ver. 1.3.4, Sable Systems International).

As experiments were performed at different elevations, the following steps were taken to ensure that results were comparable between locations. The LiCor infrared CO_2 analyzer was recalibrated using known gas mixtures before experiments at both elevations (Irvine and Mammoth Lakes). Although barometric pressure differs between these two sites, the fact that we used a mass-flow controller (which expresses flow rates in STP-corrected volumes) to push air through the insect chamber and gas analyzer ensured that the chosen flow rate of 100 mL min^{-1} at STP was maintained. Furthermore, to ensure no effect of elevation on CT_{max}, a subset of mosquitoes collected in Mammoth Lakes was tested in Irvine. No significant differences in CT_{max} were found. This is consistent with previous findings that oxygen partial pressure does not affect CT_{max} in insects except at extremely low partial pressures (2.5%; Klok et al. 2004; Stevens et al. 2010). Therefore, differences in oxygen partial pressure between the elevations used in our study were assumed to be physiologically insignificant for these animals. All results are presented for standard pressure at the temperatures indicated.

Data analyses were performed using Expedata software. $\dot{V}CO_2$ data recorded during thermolimit trials were first drift corrected and converted from parts per million to microliters of CO_2 per hour and then plotted along with the temperature data (fig. 1*A*). CT_{max} was determined using analytical procedures modified from Lighton and Turner (2004) and described in Vorhees and Bradley (2012). We defined CT_{max} as the temperature at which spiracular activity ceases, indicative of physiological failure immediately before death. Therefore, we measured the CT_{max} of each mosquito as the temperature experienced during the final 10 s of spiracular activity displayed by the insect. This value was identified using the "differentiate" and "square" functions in Expedata to magnify differences between spiracular activity and electrical noise (fig. 1*B*). The final 10 $\dot{V}CO_2$ data points before the point at which this differentiated trace leveled to 0 were then selected as the cessation of spiracular activity, and the mean temperature of these points was recorded as CT_{max}.

To determine CT_{max} from activity data, we employed techniques recommended by Lighton and Turner (2004). CT_{max} was identified as the inflection point in the absolute difference sum (ADS) or the cumulative sum of the absolute differences between adjacent data points. This method identifies the transition from high to low short-term variability in the data and thus estimates the point at which motor activity ceases. The ADS inflection was determined by selecting the 10-min interval surrounding the breakpoint in the activity ADS and fitting these data to a linear regression. The 10 highest consecutive residuals

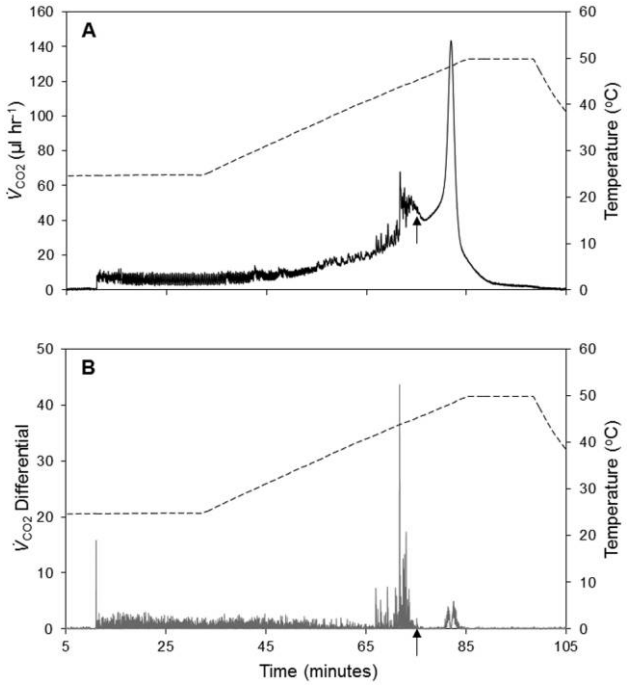

Figure 1. Representative critical thermal maximum (CT_{max}) trial in an adult female mosquito *Culex tarsalis*. *A*, $\dot{V}CO_2$ (solid line) remains stable during the equilibration phase and then increases with increasing temperature (dashed line). $\dot{V}CO_2$ reaches a plateau at 43°C in this mosquito just before the point of spiracular failure, that is, CT_{max} (arrow). *B*, $\dot{V}CO_2$ differential (solid line) for the data in *A* was calculated in Expedata to magnify differences between spiracular activity and electrical noise. The mean temperature of the 10 data points preceding where the trace levels to 0 was recorded as the CT_{max}.

of this regression were identified, and the mean temperature of these residuals was recorded as the activity CT_{max}. We calculated CT_{max} using both motor activity and spiracular activity in five samples and compared results in order to ascertain the validity of the spiracular method. As both methods provided statistically indistinguishable results ($P > 0.98$), we used only the spiracular method for all subsequent measurements.

We used temperature data collected at local weather stations located within 10 km of each site to investigate the relationship between CT_{max} values and local environmental conditions. We obtained the mean temperature and mean maximum temperature recorded at each station for the months during which mosquito collection was performed. In cases where mosquito collection spanned more than 1 mo (Coachella population), the mean and maximum values recorded for each month were averaged. To test for possible short-term thermal effects, we also collected the highest temperature recorded during the week before each collection date to examine its effects on CT_{max}. All temperature data were obtained from Weather Underground (http://www.wunderground.com; weather stations included Thermal Airport for Coachella, Rancho San Joaquin–University Park for Irvine, and Mammoth Airport for Mammoth Lakes).

Metabolic Rate

In this study, CO_2 production rate ($\dot{V}CO_2$) was used as a proxy for metabolic rate. Because mosquitoes were denied access to sugar for at least 16 h before experiments, it is likely that they were metabolizing lipids and had similar respiratory quotient values of approximately 0.7. $\dot{V}CO_2$ was measured for individuals from each site (Coachella $N = 19$; Irvine $N = 14$; Mammoth Lakes $N = 20$) using the same flow-through respirometry system described above at three ecologically relevant test temperatures: 13°, 23°, and 33°C.

Before each trial, a mosquito was placed into a 2-mL respiratory chamber inside the cabinet (but not yet inserted into the flow-through system) for 60 min at 13°C to allow the mosquito time to settle down after handling and adjust to the respiratory chamber. A baseline recording was taken for 2 min before the chamber was inserted into the airstream. Recording was paused for 2 min to allow atmospheric CO_2 to wash out after inserting the chamber. At this point the temperature profile was initiated and recording was resumed. The cabinet was programmed to hold each temperature for 45 min, always in the same order (from lowest to highest), to ensure similar thermal effects for all individuals studied. Following these trials, a subset of mosquitoes was saved and later weighed to assess differences in body mass across populations. These mosquitoes were removed from the respiratory chamber and immediately frozen in labeled 3-mL cuvettes. Frozen mosquitoes were later thawed and dried for 24 h at 70°C in a drying oven (Blue M Electric, Blue Island, IL), and dry weight was measured in milligrams using a Mettler Toledo Analytical MX5 balance accurate to 0.001 mg (Mettler Toledo, Columbus, OH). Dry weights were obtained immediately after removal from the drying oven to avoid hygroscopic water absorption.

Data analysis was performed using the Expedata software. $\dot{V}CO_2$ data were drift corrected and converted from parts per million to microliters of CO_2 per hour and then plotted along with temperature data. Metabolic rates were determined for each animal at all three test temperatures in the following way. If the mosquito exhibited resting metabolism during the final 10 min of each test temperature, metabolic rate was recorded as the mean $\dot{V}CO_2$ of that interval. If during the final 10 min of the test temperature the mosquito exhibited elevated metabolism associated with activity (i.e., flight), an earlier 10-min interval of resting metabolism was selected. If no 10-min interval of resting metabolism was available during a given test temperature, the longest interval of resting metabolism was measured. In a small number of cases where elevated metabolism persisted for the duration of the test temperature, no value was recorded.

Statistical Analyses

Data were first assessed for normality and equality of variances using a Shapiro-Wilk test and a Bartlett's test, respectively. Dry body masses were compared among populations using ANOVA. Comparisons of CT_{max} among populations were performed by

Table 2: Results from multiple regression analyses of metabolic rate (log μL CO_2 h^{-1}) against temperature (°C) and body mass (g) within each of three *Culex tarsalis* populations sampled across California

Population and variable	Slope	r^2	t	df	P
Coachella		.95	**227.31**	2, 28	<.001
Temperature	.027 ± .001		21.29	28	<.001
Mass	.149 ± .059		2.54	28	.017
Irvine		.86	**80.68**	2, 29	<.001
Temperature	.035 ± .003		11.88	29	<.001
Mass	.510 ± .114		4.50	29	<.001
Mammoth Lakes		.69	**61.98**	2, 57	<.001
Temperature	.033 ± .003		10.47	57	<.001
Mass	.210 ± .071		2.87	57	.006

Note. Values in bold are F values.

incorporating all relevant variables into a general linear model. Pearson correlation coefficients between the initial set of seven variables were examined. Latitude, altitude, and mean temperature were found to be highly correlated, and thus only mean temperature was used in our analysis to avoid multicollinearity. Mean temperature for the month of collection, mean daily maximum temperature for the month of collection, and highest temperature recorded during the week before collection were used as categorical predictors, while days postcapture was used as a continuous predictor.

Our next statistical analysis examined the effects of two continuous variables, temperature (T) and time in the lab (t), on metabolic rates using a linear mixed effects (LME) model after $\log_{10}$ transformation. In addition, the analysis examined the effects of population of origin ($i = 1$ [Coachella], 2 [Irvine], or 3 [Mammoth Lakes]) and individual ($j = 1, ..., 153$) on metabolic rate. Specifically, we assumed that metabolic rate, $y_{ij}(T, t)$, is described by the linear function

$$\alpha + \delta_i\beta_i + \gamma T + \delta_i\varphi_i T + \rho t + b_j + \varepsilon_{ijTt}, \quad (1)$$

where $\delta_i = 0$ if $i = 1$ or 1 otherwise and b_j and ε_{ijTt} are random variables with $b_j \sim N(0, \sigma^2)$ and $\varepsilon_{ijTt} \sim N(0, \sigma_e^2)$. The effects of population on the y-intercept of this linear relationship were determined by the values of β_2 and β_3, while the effects of population on the slope of the metabolic rate–temperature relationship were determined by the values of φ_2 and φ_3 (Pinheiro and Bates 2000).

Our analysis of the full model (eq. [1]) showed that ρ was not significantly different from 0. In addition, the reduced model with time in the lab effects removed had smaller Akaike and Bayesian information statistics. Accordingly, our final analysis was carried out on equation (1) with $\rho = 0$.

We also carried out pairwise comparisons of the three populations to determine whether there were significant differences among populations for the intercept and slope of the MR-T relationships. To keep the Type I error at 5% or less, we adjusted the significance level of each individual test to 0.017% (0.05/3) as prescribed by the Bonferroni inequality (Miller 1966). All statistical tests were carried out with R (R Development Core Team 2012). Throughout the text, values are represented as mean ± SEM.

Results

CT_{max} differed significantly across all three populations of *Culex tarsalis* examined (ANOVA: $F_{2,69} = 14.85$, $P < 0.001$; fig. 3). CT_{max} was highest in mosquitoes sampled from Coachella (45.98° ± 0.17°C), followed by Mammoth Lakes (45.23° ± 0.15°C), and lowest in mosquitoes from Irvine (44.47° ± 0.23°C). A general linear model showed that only mean daily maximum temperature predicted CT_{max} ($P < 0.0001$; fig. 3*A*). Mean temperature ($P > 0.90$; fig. 3*B*), highest temperature during the week before collection ($P > 0.90$; fig. 3*C*), and days spent in the lab postcapture ($P > 0.10$) did not have significant effects. Because mean temperature was highly correlated with both altitude and latitude (described in "Material and Methods"), it

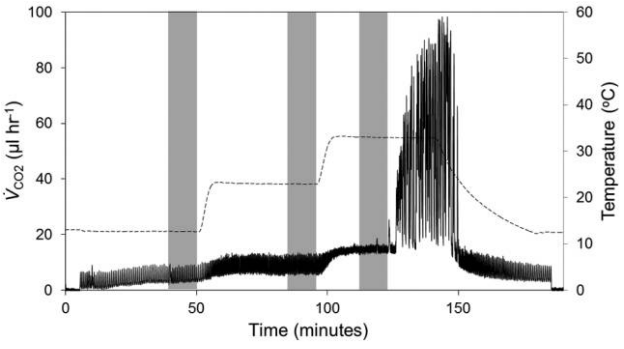

Figure 2. Representative flow-through respirometry trial in an adult *Culex tarsalis* (dry mass 1.252 mg). Metabolic rate was measured as $\dot{V}CO_2$ (solid line) at each of three temperatures (dashed line) by recording the mean $\dot{V}CO_2$ for the most recent 10-min interval of resting metabolism (bars). Periods of activity or elevated metabolism (such as between minutes 125 and 150) were omitted from analyses.

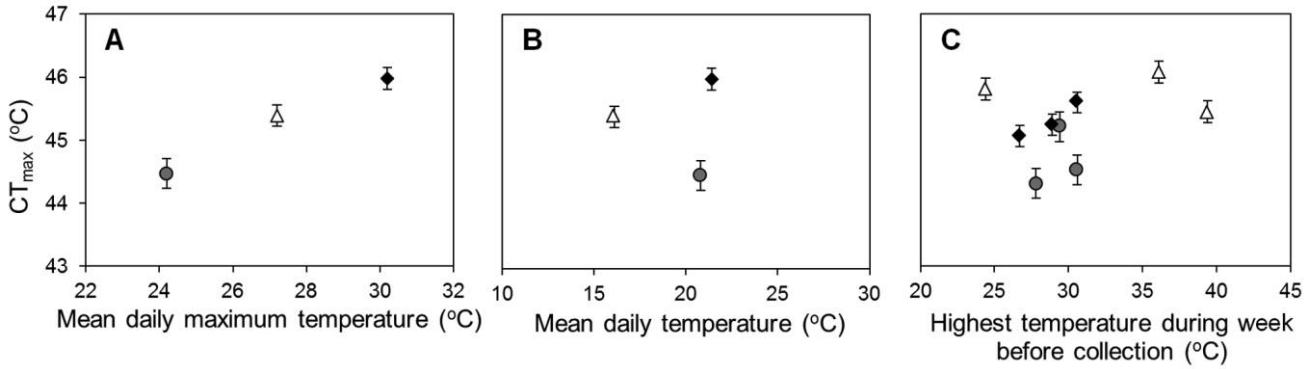

Figure 3. Critical thermal limit (CT_{max}) variation in *Culex tarsalis* populations sampled from three thermally distinct habitats in California: Coachella Valley (diamonds), Irvine (circles), and Mammoth Lakes (triangles). Mosquitoes were sampled during the season of peak abundance at each sampling site. *A*, CT_{max} (mean ± SEM) as a function of mean daily maximum temperature during the month(s) of collection. *B*, CT_{max} as a function of mean temperature at each site during the month(s) of collection. *C*, CT_{max} as a function of the highest temperature observed during the week before collection (three collection dates per population). Error bars represent SEM.

can be inferred that these variables did not have significant effects on CT_{max}.

Both individual dry body mass and temperature had significant, positive effects on metabolic rate within each population (table 2; figs. 2–4); however, dry body mass was not significantly different between populations (Coachella = 1.11 ± 0.06 mg, Irvine = 1.13 ± 0.07 mg, Mammoth Lakes = 1.19 ± 0.08 mg; ANOVA, $P > 0.70$). The slope for the MR-T relationship was converted to Q_{10} and found to be highest in the Irvine population ($Q_{10} = 2.29 \pm 0.16$), lower in Mammoth Lakes ($Q_{10} = 2.09 \pm 0.06$), and lowest in Coachella ($Q_{10} = 1.97 \pm 0.05$). An LME revealed significant slope differences between the Irvine and Coachella populations, while no differences were found between the other pairs of populations (table 3; fig. 4). The LME also showed significant differences in intercept between Mammoth Lakes and the other two populations (table 3; fig. 4).

Discussion

CT_{max}

In this study, we demonstrate that populations of a widely distributed mosquito species, *Culex tarsalis*, differ with regard to their responses to temperature across a climatic gradient. Specifically, CT_{max} differed significantly among three populations of *C. tarsalis* inhabiting climatically diverse regions. Although a number of previous studies have found variation in upper thermal limits among broadly distributed populations of insects, particularly in drosophilid flies (Hoffmann et al. 2005; Hoffmann 2010; Sgrò et al. 2010), ours is among the few to examine this phenomenon in wild populations (e.g., Klok and Chown 2003; Terblanche et al. 2006). Among researchers studying wild-caught insects, Terblanche et al. (2006) used thermolimit respirometry to examine thermal tolerance in populations of tsetse flies *Glossina pallidipes* distributed across altitudinal and latitudinal clines (Terblanche et al. 2006). CT_{max} varied least compared with other traits such as CT_{min} and water loss rates, with the largest difference in CT_{max} between any two population means being only 0.6°C. By contrast, differences between mean CT_{max} values for *C. tarsalis* populations ranged from 0.75° to 1.5°C. Although the source of these differences in magnitude is currently unclear, it is possible that our field sites are more climatically diverse than those used by Terblanche et al. (2006).

In this article, we investigated which environmental parameters were most strongly correlated with CT_{max} in *C. tarsalis* and found that CT_{max} correlated significantly with mean daily maximum temperatures. By contrast, CT_{max} did not correlate with shorter-term thermal conditions (i.e., highest temperature during the week before collection) or with the mean temperatures at these sites. This is particularly interesting given that average temperatures are commonly used in studies of geographic trait variation to infer the selective forces driving local

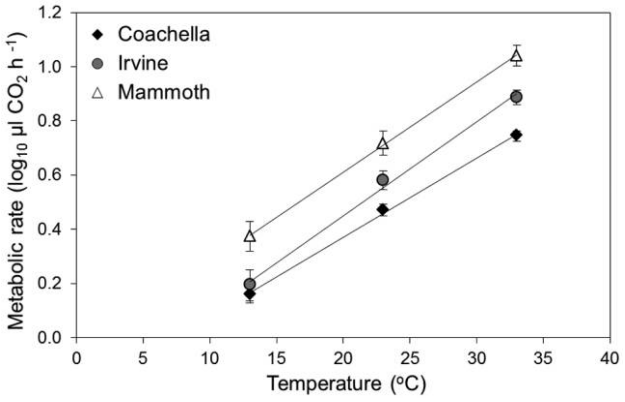

Figure 4. Log_{10} metabolic rate of *Culex tarsalis* populations from Coachella ($N = 19$), Irvine ($N = 14$), and Mammoth ($N = 20$) at 13°, 23°, and 33°C. Error bars represent SEM.

Table 3: Linear mixed-effects model testing the effects of temperature on metabolic rate in *Culex tarsalis*

Population	Intercept	Slope
Coachella	$-.220^{A}$	$.030^{A}$
Irvine	$-.249^{A}$	$.035^{B}$
Mammoth Lakes	$-.032^{B}$	$.033^{AB}$

Note. Pairwise comparisons of the three populations were done to identify significant differences for the intercept and slope of the metabolic response to temperature relationship. Different letters indicate statistically significant differences among populations within each category. Significance level was adjusted to $P = 0.017$ (0.5/3) as prescribed by Bonferroni inequality.

adaptation in thermal tolerance traits (discussed in Ragland and Kingsolver 2008). Despite this, a growing body of evidence shows that average temperatures do not in fact represent physiologically relevant metrics of environmental variability (Helmuth et al. 2010; Kingsolver et al. 2011). Helmuth et al. (2010) assert that the continued use of such variables can limit our ability to understand how climate acts to set physiological limits and that care should be taken in future studies to evaluate which environmental parameters are involved in the traits being examined. Our results provide additional evidence that average temperatures do not drive selection on thermal traits such as CT_{max} and may not be appropriate for characterizing field sites in physiological studies. In addition, CT_{max} was not correlated with the highest temperature during the week before collection. This finding supports the notion that long-term thermal conditions rather than short-term thermal effects are the underlying source of thermal differences among these populations.

Metabolic Rate

We demonstrate that the *C. tarsalis* populations tested have also diverged with regard to their MR-T relationships. Mosquitoes sampled from Mammoth Lakes showed significantly higher metabolic rates at all three test temperatures compared with the two Southern California populations. This result is consistent with other studies reporting evidence for thermal compensation in ectothermic animals inhabiting cool climates (Berrigan and Partridge 1997; Gaston and Chown 1999; Addo-Bediako et al. 2002). Mammoth Lakes is a cool alpine region that experiences the coldest temperatures of all three sites, with summer ambient temperatures reaching as low as 1°C during July 2011.

A similar study by Terblanche et al. (2009) compared the MR-T performance curves of four populations of the tsetse fly *G. pallidipes* collected across a range of climates in east Africa. Only the population from the coolest site (elevation 1,691 m) showed a significantly different slope from the other populations, and significant differences in metabolic rate (i.e., *y*-intercept) occurred only at the highest test temperature (32°C). The authors postulate that the shape of the MR-T performance curve in *G. pallidipes* may be driven by the timescale of thermal responses (short-term plasticity vs. longer-term evolved differentiation; Terblanche et al. 2009). For *C. tarsalis*, the cool-adapted population in Mammoth Lakes differed from the two other populations in *y*-intercept but not slope, while the Irvine population showed a significantly greater slope than that of Coachella. Changes in the MR-T response curve in *C. tarsalis* appear to respond to the degree of local climate variability. Specifically, populations from more variable climates (i.e., Coachella and Mammoth Lakes) showed lower thermal sensitivity compared with populations from stable climates (Irvine). This finding is consistent with previous work that has shown that insects from thermally stable environments show greater thermal sensitivity under variable thermal conditions (Angilletta et al. 2012; Nilsson-Ortman et al. 2012; Williams et al. 2012).

Role of Population Structure

A recent study of the genetic structure of *C. tarsalis* populations inhabiting North America provides an insightful context to our results. Using microsatellite data, Venkatason and Rasgon (2010) identified three genetically distinct clusters of *C. tarsalis* in the western United States, each separated by geographic and climatic barriers. In our study, we sampled *C. tarsalis* from two of these genetic clusters (Coachella and Mammoth Lakes) and from one mixed population (Irvine) that receives gene flow from both clusters. It can be predicted that if variation in either CT_{max} or metabolic rate has genetic underpinnings, differences in those traits should persist across genetic clusters. In addition, the mixed population in Irvine should exhibit traits intermediate between the adjacent populations because continual gene flow from both original clusters should prevent local adaptation.

Our results for CT_{max} do not support the hypothesis that the Irvine population exhibits characteristics intermediate between those of Coachella and Mammoth Lakes populations. The Irvine population showed CT_{max} values both distinct from and lower than those of the other two populations. This supports the hypothesis that CT_{max} is not a function of genetic relatedness but rather of local climate, specifically, the maximum temperatures experienced. Because the Irvine population inhabits a region that receives continuous gene flow from adjacent population clusters, our results suggest a significant role for phenotypic plasticity in creating population-level variation in CT_{max}. This is consistent with many previous studies of insects that have attributed population-level variation in upper thermal limits to physiological plasticity rather than to genetic differences (Ayrinhac et al. 2004; Hoffmann et al. 2005; Terblanche et al. 2006). For metabolic rate, significant differences were observed only between Mammoth Lakes and the other two populations, while metabolic rate was not different between the Irvine and Coachella populations. This result suggests that a genetic basis for metabolic rate variation in this species cannot be ruled out. Further studies using common garden experiments and multiple populations will provide further insight into these questions.

Broader Perspectives

In studying a broadly distributed species, we contribute new insights into how species may respond to climate change and which traits may be indicative of vulnerability. In this study, local temperature extremes correlated directly with CT_{max}, which supports Hoffmann's assertion that thermal extremes are more relevant than averages to understanding species responses to temperature as well as their vulnerability to climate change (Hoffmann 2010). In species with limited genetic diversity or limited potential for phenotypic plasticity, evolutionary changes in CT_{max} may not be able to keep pace with climate change. Future studies that compare plasticity in thermal phenotypes as a function of local climatic characteristics will be particularly helpful for understanding the role that thermal traits have in conferring resilience to variable climatic conditions. Particularly in the context of vector-borne diseases, understanding how thermal traits affect ecological success in vector species such as *C. tarsalis* will be critical for predicting the future distribution of human disease.

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