



# Thermal acclimation in a complex life cycle: The effects of larval and adult thermal conditions on metabolic rate and heat resistance in *Culex pipiens* (Diptera: Culicidae)



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## ABSTRACT

It has now been well established that insects can respond to variation in their environment via acclimation, yet the extent of the response varies among populations and environmental characteristics. One under-investigated theme which may contribute to this variation concerns acclimation effects across the life cycle. The present study explores how acclimation in the larval stage of *Culex pipiens* affects thermal relations in the adult stage. Mosquitoes were reared in a full factorial design at 18 or 26 °C as larvae and adults, then critical thermal maxima ( $CT_{max}$ ) and metabolic rate–temperature relationships (MR–T) were determined for all 4 treatments.  $CT_{max}$  was positively affected by both larval and adult acclimation treatments. MR–T slope was significantly affected only by adult treatment: warm acclimated adults had on average shallower slopes and higher y-intercepts than cool acclimated ones. These results demonstrate that larval acclimation effects can alter adult phenotypes in a species whose life cycle includes two drastically different environments, an aquatic and a terrestrial stage. Studying insects with complex life cycles, especially those with aquatic or subterranean larval stages, can provide valuable information on the effects of thermal variability and predictability on phenotypic plasticity.

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## 1. Introduction

Recent research concerning climate change has demonstrated that not only have average temperatures increased over the past 30 years, but also that the incidence and range of summertime extreme highs is on the rise (Fischer and Schar, 2009; Hansen et al., 2012). This phenomenon may be particularly relevant to temperate species, as thermal anomalies can be more drastic at higher latitudes (Hoffmann, 2010). The extent to which animals living in these regions will be able to cope with more extreme temperatures remains uncertain, although research has revealed a major role of adaptive phenotypic plasticity in promoting survival in the face of environmental heterogeneity (Angilletta, 2009; Bowler and Terblanche, 2008; Hoffmann et al., 2003; Huey et al., 1999; Kingsolver and Huey, 1998; Lande, 2009; Maynard Smith, 1957; Somero, 2010).

Small ectotherms, such as insects, are particularly susceptible to thermal anomalies as their body temperature closely matches environmental temperature. Since body temperature directly affects metabolic rate, performance and fitness (Angilletta, 2009; Hochachka and Somero, 2002; Huey and Kingsolver, 1989), organismal success should correlate with the ability to alter

phenotypes in response to environmental cues. For example, acclimation temperature can have major effects on various stress resistance parameters, such as cold tolerance, heat tolerance and locomotor performance (Ayrinhac et al., 2004; Fischer et al., 2010; Gibert et al., 2001; Hoffmann et al., 2005; Overgaard et al., 2011). Thermal acclimation also impacts metabolic rate (Niehaus et al., 2012; Terblanche et al., 2005) as well as the sensitivity of metabolic rate to temperature (Lachenicht et al., 2010; Terblanche et al., 2009); since metabolic rate represents the rate of biological reactions in the body, its sensitivity to thermal variation can have major implications on organismal fitness. However, research has also revealed that the extent of thermal plasticity can vary among traits measured within a population (Terblanche et al., 2006) as well as between populations for a given trait (Overgaard et al., 2011; Seebacher et al., 2012). These studies provide an uneven landscape of understanding, as many patterns have been described but the evolutionary and physiological mechanisms involved in producing these patterns are still far from resolved.

One relatively new area of research concerns quantifying acclimation effects across the life cycle (Kingsolver et al., 2011). Most insects have a complex life cycle in which larvae and adults occupy different niches and are subjected to different stresses. The vast majority of studies examining phenotypic plasticity in holometabolous insects have failed to distinguish acclimation effects experienced during the larval and adult stages. The few studies to date

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that examined the effects of developmental thermal environments on adult fitness traits have demonstrated that larval conditions affect some adult traits but not others (Ragland and Kingsolver, 2008; Terblanche and Chown, 2006) and that larval effects can decrease with adult age (Gibert et al., 2001; Zeilstra and Fischer, 2005). Therefore, research shows that thermal variation registered by the larvae transcends into the adult stage, but its effects cannot be generalized across species and traits.

*Culex pipiens* is a mosquito that occurs throughout temperate latitudes including North America and has overlapping generations during the summer. After overwintering as adults, mated females begin the cycle of bloodfeeding and ovipositing as soon as nighttime temperatures become sufficiently warm for activity. Several generations occur throughout the summer until temperatures drop and the photoperiod shortens (Spielman and Wong, 1973; Vinogradova, 2000). These holometabolous insects have a complex life cycle encompassing drastically different environments: eggs hatch into aquatic habitats where the larvae grow for up to several weeks, then following pupation, adults emerge into the terrestrial environment. As a result, individuals share their life between two distinct niches, with different food sources, different modes of locomotion and different stresses. Mosquitoes spend a potentially large portion of their life in the water, where high thermal inertia results in relatively stable conditions. Following metamorphosis, adults find themselves in the terrestrial environment and likely experience more thermal variability than they did as larvae. Because of the drastic difference in habitat, it is unclear how larval conditions influence adult thermal tolerance.

Previous research on the mosquito species *Culex tarsalis* revealed that populations occurring in different thermal environments had correlated differences in thermal tolerance (Vorhees et al., 2013). While this study demonstrated that both critical thermal maximum ( $CT_{max}$ ) and the effect of temperature on metabolic rate vary among geographically distinct populations, it did not examine the extent to which this variation was due to acclimatization versus local genetic differentiation. Broadly distributed species such as *C. tarsalis* and *C. pipiens* that can also readily disperse (Service, 1980) are valuable models for investigating evolutionary mechanisms of climate adaptation. Because they are major vectors of West Nile virus and other diseases in the US (Hayes et al., 2005), understanding the response of *Culex* mosquitoes to climate change will provide information important to predicting the future distribution of human and animal diseases.

The goals of the present study were twofold. First, I aimed to determine whether *Culex* mosquitoes can modify their thermal tolerance via acclimation. My other goal was to assess the relative effects of life stage-specific thermal acclimation on adult thermal performance in an insect that occupies sequentially two drastically different environments. The thermal performance parameters measured consist of critical thermal maximum ( $CT_{max}$ ) and metabolic rate–temperature relationship (MR–T). This investigation was performed on the mosquito *C. pipiens*, a relative of *C. tarsalis* and widespread inhabitant of central and eastern US.

## 2. Materials and methods

### 2.1. Study organisms and collection

*C. pipiens* mosquitoes were collected between mid June and mid September of 2012 in Colorado Springs, Colorado, USA, which is at an elevation of 1830 m. Individuals were collected either as eggs or first instar larvae from rain collection containers in suburban locations. Animals were returned to the laboratory at Colorado College, in Colorado Springs, CO and reared in controlled conditions.

### 2.2. Experimental treatments

Eggs and 1st instar larvae collected from the field were placed at 18 °C for 3–4 days and were then divided into replicate containers of approximately 50 larvae per 250 mL. Groups were placed in one of 2 temperature controlled incubators at either 18 or 26 °C with a 12L:12D cycle and a relative humidity of 60–70%. Throughout larval development, insects were provided daily with a 2:1 mixture of ground fish flakes and yeast. Time between first exposure to the experimental temperature and pupation was at least 12 days. Pupae resulting from these containers were placed into pint-sized emergence cups at either temperature, and emerging adults were provided with a cotton ball soaked in a 10% sucrose solution. This setup resulted in a full factorial design consisting of 4 experimental groups: L18A18 (larvae at 18 °C, adults at 18 °C), L18A26, L26A18, L26A26. Adult females were tested at 4–5 days post-emergence.

### 2.3. Measurement of critical thermal maxima ( $CT_{max}$ )

Measurements of  $CT_{max}$  were performed on adult female mosquitoes using thermolimit respirometry (Lighton and Turner, 2004). Briefly, air was scrubbed of water and CO<sub>2</sub> and passed through a 3 mL chamber containing an individual mosquito, at a constant flow rate of 100 mL min<sup>-1</sup> (mass flow controller, Sierra Instruments, Monterey, CA). Temperature of the air flowing through the mosquito chamber was monitored via a thermocouple inserted into the tubing immediately upstream of the chamber. Downstream of the chamber, the air was directed towards a gas analyzer (LiCor Li-7000, Lincoln, NE). The chamber containing the mosquito was housed inside a temperature-controlled cabinet (Pelt-5, Sable Systems International, Las Vegas, NV) programmed to remain at 25 °C for 20 min before heating up at 0.5 °C min<sup>-1</sup> for 50 min (up to 50 °C). This rate of heating was chosen according to the rationale established in Lighton and Turner (2004) and described in Vorhees et al. (2013).

Following measurement of a baseline CO<sub>2</sub> value, the mosquito was placed in the chamber and the temperature profile was initiated. At the end of each run, the dead mosquito was removed from the chamber and a second baseline was recorded. The mosquito was then dried at 70 °C for 24 h and weighed on a microbalance (Mettler-Toledo, OH, USA). Only dry mass was obtained here, as water loss from the animal at high temperature, and especially after death, likely affected body water content.

Rate of CO<sub>2</sub> production and chamber air temperature were automatically recorded using Expedata software (Sable Systems) via a universal interface (UI-2, Sable Systems) connected to the gas analyzer and the thermocouple meter (TC2000, Sable Systems).  $CT_{max}$  was determined using analytical procedures modified from Lighton and Turner (2004), and described in Vorhees and Bradley (2012). Briefly,  $CT_{max}$  was recorded as the average temperature experienced during the final 10 s of spiracular activity displayed by the insect. Because the experimental design permitted this, resting metabolic rate (MR) at 25 °C was also obtained for each mosquito. Following baseline correction, the average CO<sub>2</sub> release rate (used as a proxy for MR, see below for rationale) was obtained for a 3–5 min period within the latter half of the 20 min period preceding ramping. If the mosquito was excited during more than 5 min within that time period (identified by the lack of a regular cyclical pattern), no MR was recorded. Sample sizes for this experiment were  $N = 16$  (L18A18), 16 (L18A26), 14 (L26A18) and 14 (L26A26).

### 2.4. Measurement of metabolic rate–temperature relationship (MR–T)

Rate of CO<sub>2</sub> release was used as a proxy for MR. Because all animals had *ad lib* access to sugar water and were measured in the

same window of time (from 11 am to 2 pm) it can be assumed that all females had similar respiratory quotients. When adult female mosquitoes reached the age of 4–5 days post-emergence, 6 insects were picked from several of the four different treatments (depending on daily availability), individually placed in 3 mL respirometry chambers and connected to a multiplexor (RM-8 gas flow multiplexor, Sable Systems) which was located inside the temperature-controlled cabinet. The multiplexor allows for automated switching of the airstream between chambers. A first chamber was empty and used for measuring baseline CO<sub>2</sub> values, and the 6 mosquitoes were randomly assigned to the 6 subsequent chambers. The multiplexor was set up to cycle through a program wherein the main airstream passed through the baseline chamber for 6 min, then through each of the 6 experimental chambers for 8 min, then through the baseline chamber for 6 min again. Each cycle lasted 60 min. While the main airstream went directly from the measured chamber into the gas analyzer, the other chambers were continuously flushed with CO<sub>2</sub>-free air also at 100 mL min<sup>-1</sup>.

For each group of mosquitoes measured, the multiplexor program was set to repeat the cycle three times (for a total of 3 h). During this time, the cabinet was programmed to change temperature at a rate of 0.1 °C min<sup>-1</sup>, starting at 14 °C and ending at 32 °C. This setup resulted in each mosquito's metabolic rate being measured at 3 different temperatures separated by about 6 °C. For example, the mosquito in the first chamber was measured while ambient temperature of the airstream was at an average of 15, 21 and 27 °C; the mosquito in the last chamber was measured while ambient temperature was at 19, 25 and 31 °C. The thermocouple was inserted into the tubing of the incoming airstream to measure temperature of the air flowing into the experimental chamber for the duration of the experiment. At the end of the third cycle, mosquitoes were knocked out using CO<sub>2</sub> and weighed, then dried at 70 °C for 24 h and weighed again. Body water is reported as water mass divided by insect dry mass.

CO<sub>2</sub> release rate data recorded during each run were first drift corrected. The average CO<sub>2</sub> release rate at rest, used as a proxy for MR, was then obtained by measuring the average rate during a 3–5 min period within each 8 min period. No MR was recorded if the mosquito was excited during more than 5 min within that time period. The average temperature experienced by the mosquito during that time was also recorded and associated to the MR measurement. Only mosquitoes that exhibited measurable MR for at least 2 out of the 3 temperatures were used in data analysis. Sample sizes for this experiment were  $N = 18$  (L18A18), 25 (L18A26), 23 (L26A18) and 24 (L26A26).

### 2.5. Statistical analyses

Statistical tests were performed using SPSS version 21.0 and R version 2.15.1. Data were first tested for normality and equality of variances using Shapiro–Wilk and Bartlett's tests, respectively. Comparisons of CT<sub>max</sub> and MR values between experimental treatments were performed using a general linear model which included larval and adult temperatures as fixed effects and dry mass as a covariate.

Data from the MR–T experiment were compared using a general linear mixed effects model with larval and adult treatment temperatures as fixed effects. Test temperature was treated as a continuous variable since individual mosquitoes were measured at slightly different temperatures. To accommodate the repeated measures design of this experiment we treated individual mosquitoes as random factors. MR data were dry mass-corrected to control for body mass effects on MR (represented as mass specific MR or ms-MR). A first model was run including both 2-way and 3-way interaction effects; because the 3-way interaction was not significant the model was rerun with only 2-way interactions.

Dry mass data collected from both experiments and water content data collected from the MR–T experiment were analyzed by full factorial ANOVA to assess the effects of larval temperature, adult temperature and the interaction thereof on these parameters. For the analysis of body water content, dry mass was included in the model as a covariate.

### 2.6. Weather data

Data for mean daily maximal temperature and mean daily average temperature were obtained for the weeks of June 27–July 3 and July 4–10 for 1973–2012 in Colorado Springs, CO, from <http://www.wunderground.com>.

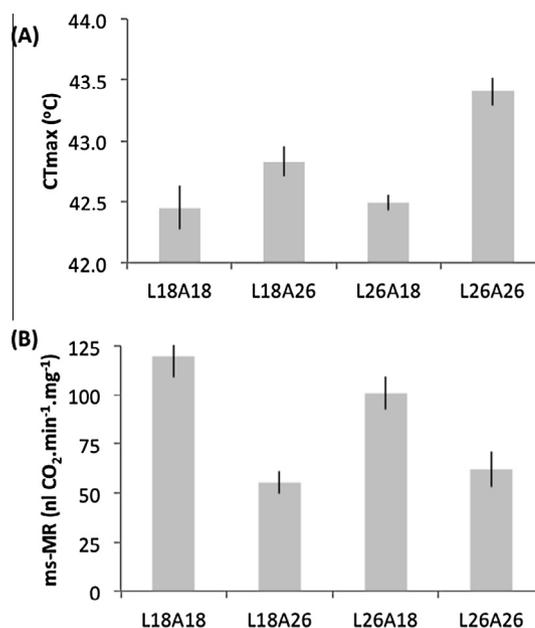
## 3. Results

Larval and adult temperatures both significantly affected the CT<sub>max</sub> of *C. pipiens*: both higher larval and adult temperatures resulted in a higher CT<sub>max</sub> (Table 1; Fig. 1A). Mosquitoes with the highest CT<sub>max</sub> were those that had been reared their entire life at 26 °C, whereas those with the lowest CT<sub>max</sub> had been reared at 18 °C. While higher adult rearing temperature resulted in higher CT<sub>max</sub> regardless of larval temperature, a higher larval rearing

**Table 1**

Results of the ANOVA testing the effects of larval and adult rearing temperatures on critical thermal maximum (CT<sub>max</sub>) and resting metabolic rate at 25 °C (MR<sub>25</sub>).

Trait	Effect	df	F value	P value
CT <sub>max</sub>	Dry mass	1	0.10	0.752
	Larval T	1	10.98	0.001
	Adult T	1	42.88	<0.001
	Larval T * Adult T	1	0.08	0.772
MR <sub>25</sub>	Dry mass	1	9.30	0.004
	Larval T	1	1.64	0.206
	Adult T	1	28.75	<0.001
	Larval T * Adult T	1	0.30	0.584

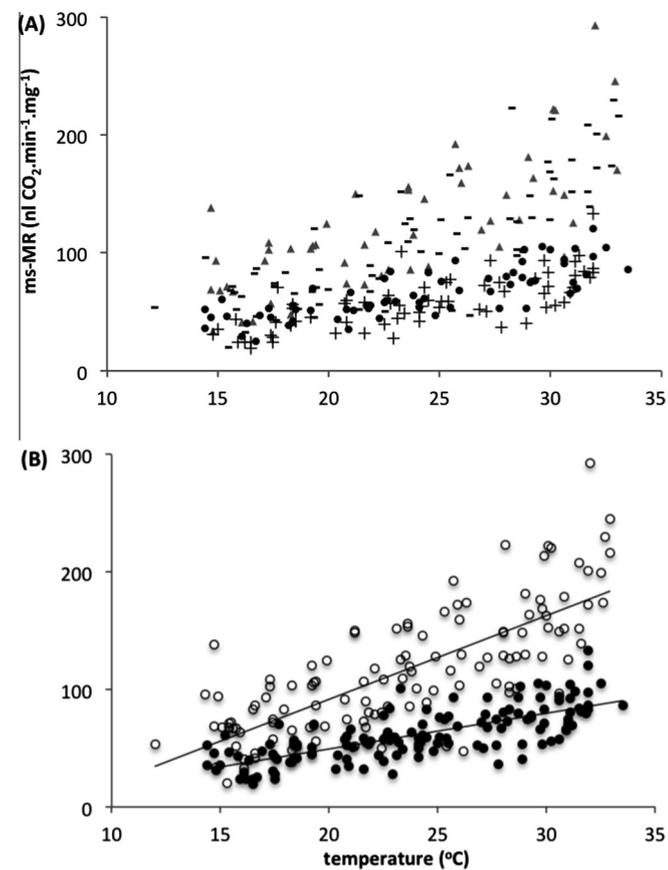


**Fig. 1.** Average (A) CT<sub>max</sub> and (B) mass-specific MR values of 4–5 day-old female *C. pipiens* reared as larvae at either 18 or 26 °C, then as adults at either 18 or 26 °C. Both larval and adult treatment temperatures significantly affected CT<sub>max</sub> whereas only adult temperature significantly affected mass-specific MR (see Table 1 for details). Bars represent S.E.

temperature seemed to only affect  $CT_{max}$  when adults were acclimated to 26 °C. However, the interaction effect between larval and adult rearing temperatures was not significant. In the process of collecting  $CT_{max}$  data, the animals were subjected for a period of time to 25 °C and their resting MR was determined. It was found that body mass and adult rearing temperature affected MR, but that larval rearing temperature did not (Table 1; Fig. 1B).

Examination of the MR–T relationships revealed significant effects of adult temperature treatment on both the y-intercept and the slope of the relationship (Fig. 2A and B; Table 2). However, larval rearing temperature had no effect on the MR–T relationship. Animals reared at the higher temperature as adults had on average shallower MR–T slopes, and within the range of temperatures tested they had a lower MR at any given temperature than animals reared at low temperature as adults.

Mosquito mass was affected by both larval and adult temperatures: lower larval temperature and higher adult temperature resulted in larger mosquitoes (Table 3; Fig. 3A). However, there was no significant interaction effect. Body water content was significantly affected by adult but not larval temperature, such that adults reared in the warmer environment had less body water per unit dry mass than did those reared in the cooler environment (Table 3; Fig. 3B).



**Fig. 2.** Mass-specific resting metabolic rate values of 4–5 day old female *C. pipiens*; each mosquito was tested at 3 temperatures. (A) raw data including all mosquitoes at all temperatures; markers represent L18A18 ( $\blacktriangle$ ), L18A26 (+), L26A18 ( $-$ ), L26A26 ( $\bullet$ ). (B) same data, but for each adult treatment group both larval treatments were pooled together (since larval temperature was found to have no significant effect on the outcome); markers represent A18 (open circles) and A26 (closed circles). Lines are ordinary least squares regression lines. Statistical comparisons are detailed in Table 2.

**Table 2**

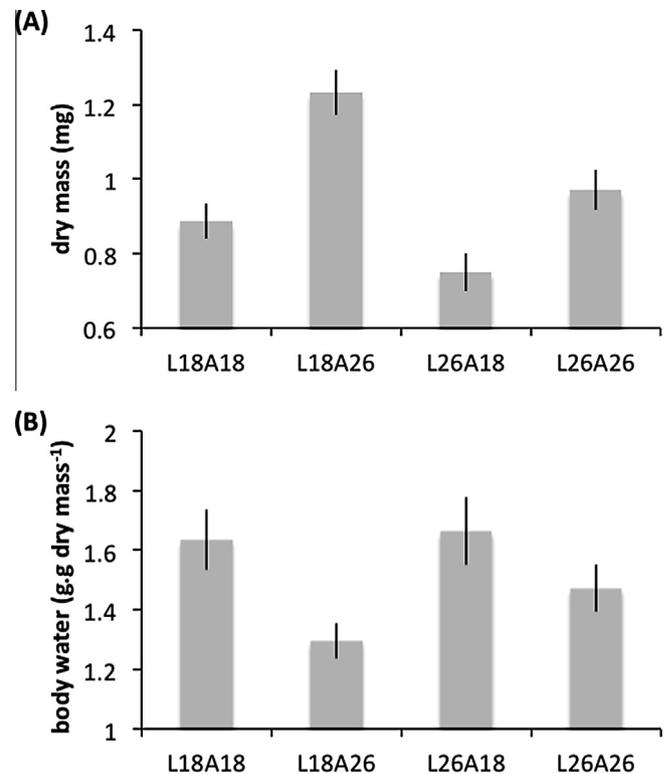
Results from the linear mixed effects model testing the effects of larval and adult treatment temperatures and test temperature on the mass-specific resting metabolic rate (ms-MR) of *C. pipiens*. Because each mosquito was measured at 3 temperatures, mosquito was treated as a random unit. Only results from the model including the 2-way interactions are shown, since the 3-way interaction was not significant.

Trait	Effect	df	t value	P value
ms-MR	Intercept	167	-2.02	0.045
	Test T	167	8.163	<0.001
	Larval T	87	-0.712	0.479
	Adult T	87	3.370	0.001
	Larval T * Test T	167	0.530	0.597
	Adult T * Test T	167	-5.893	0.005

**Table 3**

Results of the ANOVA testing the effects of larval and adult rearing temperatures on dry body mass and dry mass-specific body water content of 4–5 day old female *C. pipiens*.

Trait	Effect	df	F value	P value
Dry mass	Larval T	1	13.53	<0.001
	Adult T	1	27.34	<0.001
	Larval T * Adult T	1	1.38	0.242
ms-Body water	Larval T	1	0.14	0.704
	Adult T	1	17.67	<0.001
	Larval T * Adult T	1	2.85	0.095



**Fig. 3.** Body characteristics of 4–5 day old female *C. pipiens*. Both larval and adult treatment temperatures significantly affected dry mass (A) whereas only adult temperature significantly affected body water (B) (see Table 3 for details). Bars represent S.E.

#### 4. Discussion

The present study sheds light on the phenotypic plasticity of thermal traits in a broadly distributed mosquito species. *C. pipiens* exhibited variation in both  $CT_{max}$  and MR–T relationships as a result of thermal acclimation. A recent study compared the thermal

traits of geographically distinct populations of *C. tarsalis*, a species closely related to *C. pipiens* (Vorhees et al., 2013). They found  $CT_{max}$  to differ significantly among populations by up to 1.5 °C. Similarly, MR–T relationships varied in slope and y-intercept, with populations from less variable environments showing higher sensitivity, and populations from warmer environments showing a right shift in the relationship (Vorhees et al., 2013). The present study demonstrates that at least a portion of the variation in thermal traits that was found between those populations may be due to phenotypic plasticity. The population structure of *C. tarsalis*, represented by distinct clusters exhibiting gene flow within but not between clusters (Venkatesan and Rasgon, 2010), makes this species and others like it particularly informative in studying the relative roles of phenotypic plasticity and adaptation to setting thermal tolerance traits. Considering the ability of mosquitoes to migrate several kilometers each day (Reisen and Reeves, 1990) and be transported much further by the wind (Service, 1980), gene flow between local populations living in different thermal environments can be extensive. Therefore, phenotypic plasticity is likely to play a large role in allowing individuals to survive in a variety of unpredictable habitats.

It is now well established that acclimation plays an important role in modulating insect thermal tolerance (Arias et al., 2011; Chown and Nicolson, 2004; Hoffmann et al., 2003; Kingsolver and Huey, 1998; Terblanche et al., 2006). However, the manner in which acclimation functions to set thermal tolerance across life stages has received comparatively little attention (Gibert et al., 2001; Kingsolver et al., 2011; Terblanche and Chown, 2006; Zeilstra and Fischer, 2005). Many insects have complex life cycles, in which each life stage may occupy different habitats and experience different environmental stresses. Mosquitoes, for example, live in water during the immature life stages, but are airborne as adults. Considering the major habitat differences, it is unclear whether conditions experienced during the aquatic life stage can affect the thermal physiology of the terrestrial one. Indeed, the present study demonstrates that thermal acclimation effects in *C. pipiens* larvae modulate the thermal response in the adults.

Studies examining the effects of acclimation across life stages on adult heat tolerance have reported mixed results. Maynard Smith (1957) acclimated *Drosophila buzzatii* larvae and adults to 2 temperatures and found, as in the present study, that both affected adult heat tolerance. However, Terblanche and Chown (2006) found no effect of larval rearing temperature on adult heat tolerance of the tsetse fly *Glossina pallidipes*. Similarly, no effect of rearing temperature on adult thermal tolerance was found for the mosquito *Wyeomyia smithii* (Ragland and Kingsolver, 2008). A possible explanation for these differences may reside in the extent of thermal variability and predictability experienced by larvae occupying different natural habitats. Theoretical models and experimental evidence show that phenotypic plasticity is generally favored in variable but predictable environments (Gavrillets and Scheiner, 1993; Levins, 1963; Moran, 1992; Reed et al., 2010; Scheiner, 1993). *W. smithii* larvae are typically found in pitcher plant leaves, which represent much smaller volumes of water than typical *C. pipiens* habitats – the resulting higher thermal variability of such habitats might make temperature an unreliable cue of the conditions that will be experienced by the adults. In *C. pipiens* aquatic habitats, short term unpredictable fluctuations in weather are dampened by the larger water volume such that water temperature is a more reliable predictor of the average conditions adults might face.

Similarly, larval aquatic environments may provide more reliable thermal cues on which phenotypic plasticity can act than conditions experienced in the terrestrial environment by adults. Kingsolver and Huey (1998) investigated environmental predictability to determine whether thermal plasticity in pierid butterfly

wing melanization was adaptive. They found that mean  $T_{max}$  in a given week explained less than 7% of the variation 2 weeks later (time between melanization and adult emergence); they concluded that the thermal environment experienced by larvae was a poor predictor of adult conditions. A survey of 40 years of weekly average thermal maxima in Colorado Springs (Fig. 4A) shows that variance in mean  $T_{max}$  in 1 week explains only about 3% of the variance during the following week. However, weekly thermal averages yield higher predictability from week to week (25%; Fig. 4B). While thermal conditions may be fairly unpredictable in the terrestrial environment, the damping of variability resulting from life in water may result in favorable conditions for larvae to evolve thermal plasticity traits advantageous to the adults. Determining whether this degree of short-term predictability is sufficient to drive the evolution of plasticity in  $CT_{max}$  of *C. pipiens* larvae will require assessing the fitness advantages of phenotype–environment matching against its costs (Reed et al., 2010).

Comparison of MR–T relationships among populations provides information on two major aspects of thermal biology. Changes in the slope of the relationship represent variations in thermal sensitivity, i.e. the degree to which metabolic rate responds to temperature changes (Chown and Nicolson, 2004). Shifts in y-intercept for a given slope represent temperature compensation, an up- or down-regulation of metabolic rate in response to a decrease or increase in ambient temperature, respectively (Clarke, 1993; Scholander et al., 1953). This study revealed a significant effect of

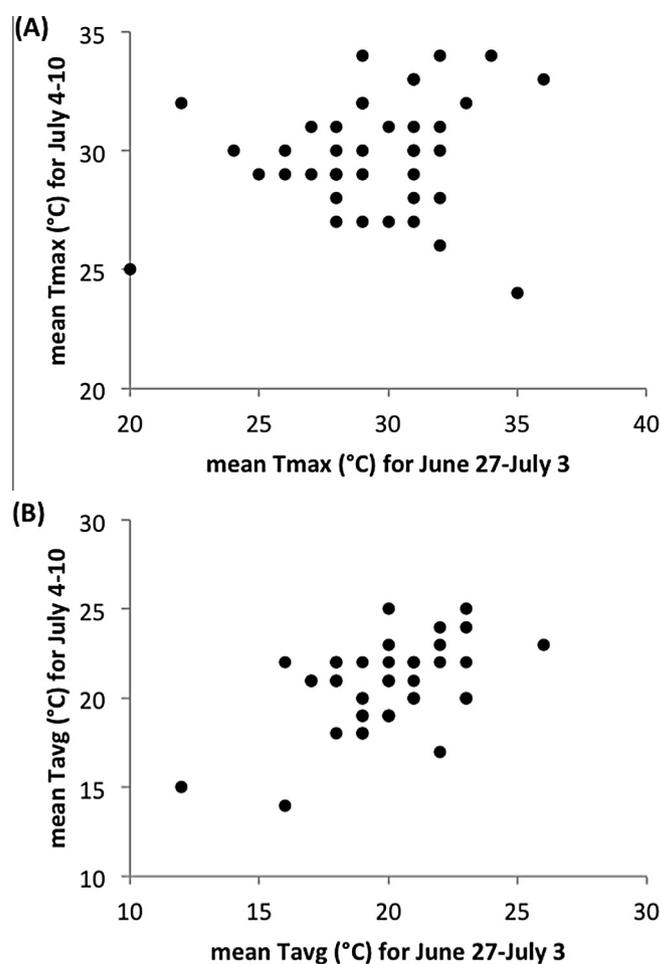


Fig. 4. Correlation of mean daily maximum temperature (A) and mean daily average temperature (B) for the weeks of June 27–July 3 and July 4–10 for 1973–2012, in Colorado Springs, CO (data from <http://www.wunderground.com>).

adult, but not larval, temperature on the thermal sensitivity of *C. pipiens*. As well, thermal compensation occurred in response to adult temperature acclimation. Adults reared at the lower temperature had on average a steeper slope, i.e. had higher thermal sensitivity, than those reared at the higher temperature. Such an increase in slope has been demonstrated for cold-adapted species or populations in the context of metabolic cold adaptation (see Addo-Bediako et al., 2002; Terblanche et al., 2009). However, thermal sensitivity was not affected by acclimation in the tsetse fly *G. pallidipes* (Terblanche et al., 2009). The authors hypothesized that the slope of the relationship might only be affected by long-term genetic change, whereas the *y*-intercept can be changed by acclimation. The present study demonstrates that, at least in *C. pipiens*, acclimation can affect both the slope and *y*-intercept of the relationship.

Evidence for temperature compensation was observed in response to adult acclimation both in the MR–T experiment and by examining the MR data collected at 25 °C. Warm acclimated adults had on average significantly right-shifted MR–T curves and significantly lower MR at 25 °C than their cold-acclimated counterparts, consistent with the phenomenon of temperature compensation (Scholander et al., 1953). These results are in agreement with Terblanche and Chown (2006) who found an effect of adult temperature but not developmental temperature on MR in adult tsetse flies. Temperature compensation of metabolic rate via acclimation has already been demonstrated in a number of insect species (Chown and Gaston, 1999; Clarke, 1993) and the present study establishes this in a mosquito species.

Larval and adult rearing temperatures had opposite effects on mosquito body mass: higher larval temperatures led to lower mass whereas higher adult temperatures led to larger mass. The effect of larval temperature on body mass in insects is consistent with the temperature-size rule (Atkinson and Sibly, 1997; Zuo et al., 2012). The effect of adult temperature on the other hand suggests that females living in the warmer environment as adults gained more weight, likely by enhanced accumulation of fat, glycogen and/or metabolically active tissues (Briegel, 2003; Gray and Bradley, 2003). In temperate mosquitoes such as *C. pipiens*, rearing temperature and photoperiod cooperatively affect the developmental trajectory of females: low temperature combined with short day-length result in the induction of diapause (Spielman and Wong, 1973). Benoit and Denlinger (2007) found that adult female *C. pipiens* reared in diapause inducing conditions had higher body mass than those reared in non-diapause conditions. Because the photoperiod used in the present study (12L:12D) had the potential to induce diapause in the cool acclimated population (Spielman and Wong, 1973), it is possible that higher mass seen in the cool acclimated larval groups may be associated with diapause physiology. However, the reason that adults placed at the higher temperature would gain more weight is unclear; perhaps this higher temperature promotes increased sugar ingestion and energy storage. In these temperate species, photoperiod and temperature have interactive effects on insect physiological systems that certainly complicate the temperature-size rule (Cabanita and Atkinson, 2006; Nakamura, 2002). Body water content was affected only by adult temperature, and variation in this may have been a side-effect of mass differences resulting from the differential accumulation of energy stores. It is also possible that variation in relative humidity in each chamber, which was maintained at around 60% RH but was not tightly controlled, may have influenced body water content of the individuals.

Similar to *C. tarsalis*, *C. pipiens* occur over a wide variety of thermal habitats in North America, and likely experience extensive gene flow only restricted by a few geographic barriers (Venkatesan and Rasgon, 2010). This study demonstrates that both *C. pipiens* adults and larvae respond to thermal acclimation, which likely

contributes to their ability to thrive in such diverse environments. However advantageous this may be in a widespread species, phenotypic plasticity can also be unfavorable in unpredictable thermal conditions such as those occurring in temperate latitudes from day to day or year to year. For organisms who spend a portion of their life cycle in thermally insulated environments such as water or soil (Kingsolver et al., 2011), it is possible that the dampened short term variation may provide information useful to predicting the thermal conditions that the adult will emerge into. Expanding the study of acclimation effects across complex life cycles is integral to further our understanding of how environmental variability and predictability modulate the evolution of phenotypic plasticity in populations.

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