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# Effects of *in situ* climate warming on monarch caterpillar (*Danaus plexippus*) development

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Climate warming will fundamentally alter basic life history strategies of many ectothermic insects. In the lab, rising temperatures increase growth rates of lepidopteran larvae, but also reduce final pupal mass and increase mortality. Using *in situ* field warming experiments on their natural host plants, we assessed the impact of climate warming on development of monarch (*Danaus plexippus*) larvae. Monarchs were reared on *Asclepias tuberosa* grown under 'Ambient' and 'Warmed' conditions. We quantified time to pupation, final pupal mass, and survivorship. Warming significantly decreased time to pupation, such that an increase of 1° C corresponded to a 0.5 day decrease in pupation time. In contrast, survivorship and pupal mass were not affected by warming. Our results indicate that climate warming will speed the developmental rate of monarchs, influencing their ecological and evolutionary dynamics. However, the effects of climate warming on larval development in other monarch populations and at different times of year should be investigated.

1 **Effects of *in situ* climate warming on monarch caterpillar (*Danaus plexippus*) development**

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25 **Running Header:** Lemoine et al: Warming effects on monarch development

## Abstract

Climate warming will fundamentally alter basic life history strategies of many ectothermic insects. In the lab, rising temperatures increase growth rates of lepidopteran larvae, but also reduce final pupal mass and increase mortality. Using *in situ* field warming experiments on their natural host plants, we assessed the impact of climate warming on development of monarch (*Danaus plexippus*) larvae. Monarchs were reared on *Asclepias tuberosa* grown under ‘Ambient’ and ‘Warmed’ conditions. We quantified time to pupation, final pupal mass, and survivorship. Warming significantly decreased time to pupation, such that an increase of 1° C corresponded to a 0.5 day decrease in pupation time. In contrast, survivorship and pupal mass were not affected by warming. Our results indicate that climate warming will speed the developmental rate of monarchs, influencing their ecological and evolutionary dynamics. However, the effects of climate warming on larval development in other monarch populations and at different times of year should be investigated.

**Keywords:** temperature, pupal mass, survivorship, climate change, growth

## Introduction

Modified temperature regimes caused by climate change may fundamentally alter insect life cycles. Indeed, many aspects of lepidopteran larval development exhibit considerably temperature-dependence. For many species, warming increases growth rates and survivorship; however both growth and survival decline rapidly once temperatures exceed an individual's thermal optimum (Kingsolver et al. 2006, Kingsolver and Woods 1997). To date, most temperature manipulation experiments have been conducted in highly controlled lab settings. Such laboratory experiments overlook natural temperature fluctuations that affect larval development and survival (Zalucki 1982) and which may not accurately reflect real climate warming patterns. Furthermore, lab experiments often use artificial foods (Kingsolver et al. 2006, Lee and Roh 2010) or leaf material that was not grown under elevated temperatures (Lemoine et al. 2014). Given that plant nutritional quality also changes under warming (Veteli et al. 2002), extrapolating results from laboratory experiments is potentially misleading. Few studies address how realistic climate warming influences lepidopteran development using *in situ* field warming experiments that simultaneously warm both insects and their host plants while realistically mimicking climate warming.

Monarchs (*Danaus plexippus*) are a charismatic species found throughout North America and are well-known for their annual migrations between Mexico and the Great Lakes region. To date, monarch migrations have been extensively studied, focusing on factors that influence migration success and population size (Reppert et al. 2010, Flockhart et al. 2015), potential overwintering and migratory habitat loss (Oberhauser and Peterson 2003, Pleasants and Oberhauser 2012, Sáenz-Romero et al. 2012), and overwintering behavior (Masters et al. 1988). Climate change has shifted research focus towards thermal constraints on monarch migration and

development; cool night time temperatures induce reproductive diapause in adult monarchs (Goehring and Oberhauser 2002, Guerra and Reppert 2013) and spring droughts reduce monarch population sizes in their summer breeding grounds (Zipkin et al. 2012). Thus, climate change may have considerable negative effects on monarch populations by reducing available overwintering and migratory habitat.

In addition to indirect effects on monarchs via habitat loss, climate change might directly alter monarch physiology. Monarch larval growth, consumption, and mortality rates all depend upon environmental temperatures (Zalucki 1982, Goehring and Oberhauser 2002, York and Oberhauser 2002, Lemoine et al. 2014). For example, prolonged exposure to extreme heat in reduces larval survival rates in laboratory experiments (Zalucki 1982, York and Oberhauser 2002). However, laboratory experiments often use extreme temperature regimes that monarchs may not encounter during their lifetime and lie outside the normal temperature range experienced by monarchs. Furthermore, warming can alter the nutritional quality of monarchs' milkweed host plants (Couture et al. 2015), yet few studies consider concurrent effects of warming on both monarch and milkweed (*but see* Couture et al. 2015). Indeed, studies that expose both monarchs and milkweed to realistic climate change scenarios that include diel and daily temperature fluctuations remain rare.

Here, we report results from an *in situ* warming experiment designed to assess how climate warming influences growth, survival, and development of monarch larvae. We hypothesized that warming would reduce larval development time, as has commonly been reported for monarch larvae (Zalucki 1982), but would also decrease pupal mass and survivorship (Zalucki 1982, York and Oberhauser 2002).

## Methods

All experiments were conducted at the Smithsonian Environmental Research Center in Edgewater, MD. Over the summer of 2013, sixteen 2 x 2 m garden beds were installed in an open field. In each plot, 1 m long aluminum sheets were driven into the soil to quarter the plot into four 1 x 1 m subplots. Two subplots were used for other experiments. The two remaining subplots were seeded with *Asclepias tuberosa* in the fall of 2013. Warming treatments were imposed by installing a single Kalglo MRM-1215 1500W (Kalglo Electronics Company, Bethlehem, PA) heater over half of the plots. An aluminum frame of the same shape and size as the heaters was hung over each control plot to mimic any shading effects ( $n = 8$  per temperature treatment). Heaters were suspended ~1.5 m from the soil surface. In October 2013, after the end of the growing season, heaters were turned off and *A. tuberosa* overwintered under natural conditions. At the beginning of the 2014 growing season, heaters were turned on and remained on throughout the experiment. *Asclepias tuberosa* was therefore germinated and grown under warming treatments for two growing seasons.

Temperature data loggers (Onset HOBO loggers) in each plot recorded average daytime temperatures of  $25.2 \pm 1.4^{\circ}\text{C}$  and average nighttime temperatures of  $19.9 \pm 2.0^{\circ}\text{C}$  in ambient conditions. Maximum daytime temperatures averaged  $30.7 \pm 2.5^{\circ}\text{C}$ , while minimum nighttime temperatures averaged  $18.2 \pm 2.3^{\circ}\text{C}$ . Since infrared heaters do not warm the air but instead warm surfaces, we verified heating treatments using a handheld IR thermometer (Kimball et al. 2008). Nighttime IR gun measurements verified that heaters raised surface temperatures by  $\sim 4^{\circ}\text{C}$  on average ( $p < 0.001$ ), which is below severe projections of a  $6^{\circ}\text{C}$  increase in temperature but above the more conservative estimate of a  $2^{\circ}\text{C}$  temperature increase by 2100 (IPCC 2007).

108 In August 2014, monarch eggs and larvae were gathered from *A. syriaca* within nearby  
109 old-growth fields. Eggs and larvae were reared in mesh cages and fed fresh *A. syriaca* leaves  
110 daily until they reached the third instar. Larval development was checked continuously  
111 throughout the day. Immediately after molting to the third instar, larvae were randomly assigned  
112 to a temperature treatment ('Ambient', 'Warmed') and placed on a single *A. tuberosa* within a  
113 randomly chosen plot ( $n = 15$ ,  $n = 18$  for 'Ambient' and 'Warmed' treatments, respectively). A  
114 mesh bag was placed over the plant to retain the monarch. First or second instar larvae escaped  
115 the mesh bags easily and thus were not used. If the monarch consumed the entire host plant, they  
116 were transferred to another plant within the same subplot. Time to pupation was recorded as the  
117 number of hours between experiment initiation and onset of chrysalis formation, and this number  
118 was converted to number of days (development hour / 24). Dead individuals were recorded and  
119 removed from the host plant. Chrysalids were carefully transported back to the lab and weighed  
120 to obtain final pupal mass.

121 We measured three plant traits (specific leaf area (SLA), water content, and latex  
122 production) to determine whether warming effects on monarch development might be mediated  
123 through warming effects on plant traits. At the end of the experiment, two newly expanded  
124 leaves were collected from each plant. For one leaf, we measured leaf area, obtained a fresh wet  
125 mass, and then dried the leaf to obtain a dry mass. We calculated specific leaf area (SLA) as area  
126 / dry mass and percent water content as  $(1 - \text{dry mass (g)} / \text{fresh mass (g)}) * 100$ . Using the second  
127 leaf, we determined latex production by cutting the tip of the leaf and blotting all latex onto a  
128 dry, pre-weighed piece of filter paper. The filter paper was dried again and latex concentration  
129 calculated as the difference in post- and pre-latex filter weights divided by leaf area (Agrawal  
130 2005).



Although heaters raised temperatures of 'Warmed' plots by  $\sim 4^{\circ}\text{C}$  on average, plots varied considerably in temperature due to different light levels across the experimental garden and varying plant biomass within each plot. We therefore measured temperature with a handheld infrared thermometer in each subplot during the night at the end of the experiment. For consistency, we recorded temperature of a white plastic sphere mounted 0.5 m from the ground in the middle of each subplot. We then treated temperature as a quantitative, rather than categorical, variable in all analyses. Note that these measures reflect relative differences in temperature among plots that should be relatively constant over the experiment.

We regressed all response variables against night-time temperatures as measured by the IR gun using OLS regressions. We regressed mortality against temperature using logistic regression, where the response variable was dichotomous with survival = 0 and dead = 1. Although monarchs experience mortality as pupae, brief exposure to prolonged temperatures did not alter pupal mortality rates and third instar individuals were the most sensitive to temperature increases (York and Oberhauser 2002). Thus, our experiment likely captured most of the influence of temperature on larval survival.

Model assumptions were verified with residual plots where appropriate. All analyses were conducted using Python v2.7 with the '*numpy*', '*pandas*', and '*statsmodels*' modules (McKinney 2010, Seabold and Perktold 2010, Walt et al. 2011).

## Results

Time to pupation declined rapidly with increasing temperature ( $p < 0.001$ ,  $R^2 = 0.57$ ) (Fig. 1). At the lowest temperature,  $12.6^{\circ}\text{C}$ , monarchs required  $12.5 \pm 0.24$  days to transition between third instar and pupa. At the warmest temperature,  $17.3^{\circ}\text{C}$ , third instar monarchs

required only  $10.3 \pm 0.2$  days to reach pupation. The slope was  $-0.46 \pm 0.08$ , suggesting that  $1^\circ \text{C}$  of warming reduces time to pupation by roughly half a day. Thus, in future climates, time to pupation may be reduced by 1 – 3 days, depending on location and severity of warming. Air temperature measurements do not accurately reflect the intensity of infrared heating because infrared energy warms surfaces and not the air (Kimball et al. 2008), calculations of degree-days may not accurately reflect the underlying temperature treatments. Still, we calculated the number of degree days experienced by each individual for which there was adequate temperature data following the simple averaging method, since temperatures remained within the upper and lower thermal limits throughout the experiment (Allen 1976). Monarch caterpillars experienced  $\sim 155 \pm 17$  degree days, and this did not differ between temperature treatments ( $p = 0.978$ ). Thus, monarchs accumulated their required number of degree days faster in the warming treatment than in the ambient treatment.

Temperature had no effect on pupal mass ( $p = 0.454$ ,  $R^2 = 0.023$ , Fig. 2). Similarly, mortality was low throughout the experiment (18%) and independent of temperature ( $p = 0.610$ , pseudo- $R^2 = 0.01$ , Fig. 3).

Warming had no effect on any measured plant trait. SLA ( $p = 0.940$ ,  $R^2 = 0$ ), percent water content ( $p = 0.313$ ,  $R^2 = 0.05$ ), and latex concentration ( $p = 0.739$ ,  $R^2 = 0.01$ ) all did not vary with temperature. Thus, any effects of warming on monarch development time were direct effects of temperature on monarch physiology rather than being mediated through the plant traits we measured.

## Discussion

Our study indicates that climate warming will accelerate monarch larval development but likely have little effect on larval mortality or pupal mass at our study site. This is consistent with numerous studies showing positive correlations between larval development and temperature (Kingsolver and Woods 1997). Since warming increases larval growth rates, lepidopteran larvae reach critical mass needed for pupation earlier and proceed through larval stadia more quickly. This is demonstrated by the fact that monarch larvae developed more rapidly but experienced roughly the same number of degree days. Our results suggest that climate warming might actually facilitate monarch development under moderate climate change scenarios at sites with relatively cool temperatures, potentially increasing the number of generations in the temperate summer breeding grounds of eastern migratory monarch populations.

Laboratory studies have consistently documented negative effects of extreme temperatures on monarch caterpillar development and survival. Short-term, extreme heat stress can have weak negative effects on pupal mass (York and Oberhauser 2002). Likewise, constant temperatures above 28° C induced high mortality rates in monarch larvae (Zalucki 1982, York and Oberhauser 2002). However, these studies used either pulses of extremely high temperatures (*i.e.* 36° C) or held monarchs at a constant temperature (*i.e.* 28° C). Ambient, maximum daytime temperatures averaged 30 °C during our experiment; warming increased this maximum to 32-34° C. Although these temperatures are above the thermal optimum of monarch survival, we found no effect of *in situ* warming on either pupal mass or survival. As temperatures exceeded 28° C for less than 20% of the full 24 hour day, it is likely that diel and daily temperature fluctuations mitigated the lethality of high temperatures.

Interestingly, our study site had warmer temperatures during our experiment than other locations of the monarch breeding range. Monarchs typically experience cool temperatures

during their northward migration: maximum March temperatures in Texas average  $23.5 \pm 2.4^\circ \text{C}$ , maximum April temperatures in Iowa and the midwestern US average  $20.7 \pm 1.5^\circ \text{C}$ , and maximum May temperatures in the Great Lakes region average  $18 \pm 2.3^\circ \text{C}$  (averages based on 50 year weather station data provided by WorldClim). Even maximum temperatures during the summer breeding season in the Great Lakes region are typically lower than at our study site, averaging  $26.0 \pm 2.3^\circ \text{C}$  compared to  $30.7 \pm 2.5^\circ \text{C}$  at during our experiment. Thus, our study site represents the upper thermal limits monarchs experience during their migrations and breeding season.

Climate change can also alter foliar water content, nutritional quality, and secondary metabolite concentrations (Zvereva and Kozlov 2006, Couture et al. 2015). However, we found little effect of temperature on *A. tuberosa* traits. Indeed, temperature often has negligible effects on secondary metabolites and nutritional content (Aerts et al. 2009, Veteli et al. 2002, Williams et al. 2000). Thus, effects of climate change on monarch development time appear related to direct effects of temperature on monarch physiology, rather than any change in host plant quality.

Although our results suggest that warming may minimally impact monarch larvae older than the third instar in temperate regions, climate change still poses a considerable threat to monarch populations. For example, increased incidence of drought may reduce the availability of *Asclepias* host plants during the northward migration, decreasing the population size of eastern migratory monarchs (Zipkin et al. 2012). Climate warming may also delay initiation of reproductive diapause in the fall, advance the cessation of reproductive diapause in the spring, and potentially cause monarchs to migrate further south than ordinary, missing their overwintering habitat or migrating north later in the year (Goehring and Oberhauser 2002,

222 Guerra and Reppert 2013). Climate warming will also increase the incidence of freezing rains  
223 during the overwintering period, leading to increased adult mortality in overwintering  
224 populations (Oberhauser and Peterson 2003). Furthermore, warming may have strong effects on  
225 monarch larvae in more tropical environments or earlier in the season. Thus, researchers and  
226 conservationists must understand how climate change will affect all parts of the monarch life  
227 cycle in order to protect this important species.

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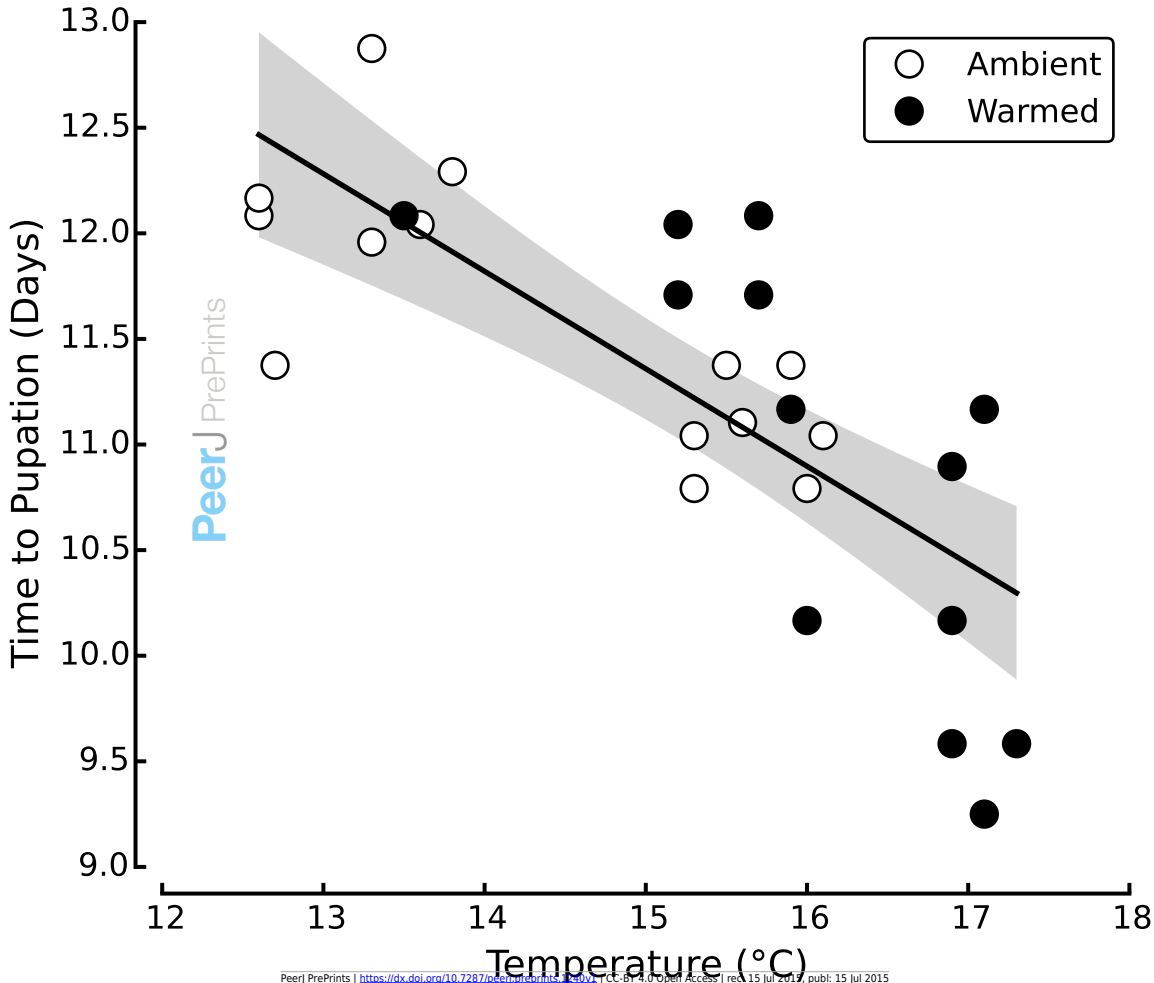


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## Figure 1 (on next page)

Effects of temperature on monarch time to pupation.

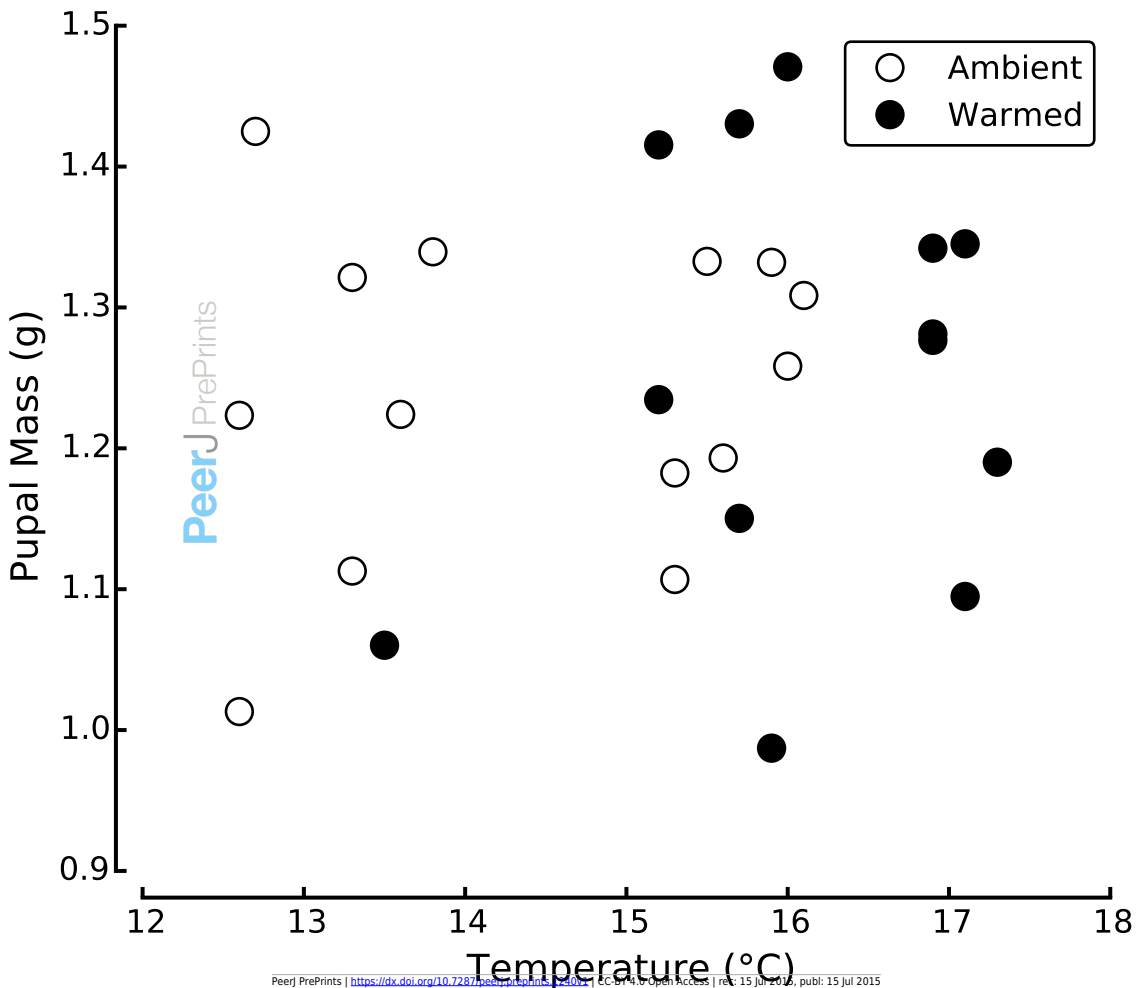
Time to pupation (in days) declined rapidly with increasing temperature. White points show individuals from the 'Ambient' plots, black points show individuals from the 'Warmed' plots. Line gives the predicted values, shaded area shows the 95% confidence interval of the fitted values.



## Figure 2 (on next page)

Effects of temperature on pupal mass.

Pupal mass was unaffected by temperature. White points show individuals from the 'Ambient' plots, black points show individuals from the 'Warmed' plots.



### **Figure 3**(on next page)

Effects of temperature on monarch survival.

Probability of mortality was not affected by temperature. A value of 0 on the y-axis indicates that the individual survived, while a value of 1 indicates that the individual died. Points have been jittered slightly to avoid overlap. White points show individuals from the 'Ambient' plots, black points show individuals from the 'Warmed' plots.

Probability of Mortality

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- Ambient
- Warmed

Temperature (°C)