

Potential impacts of climate change on monarch butterflies, *Danaus plexippus*

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ABSTRACT

Monarch butterflies are believed to be vulnerable to the effects of climate change. Ecological niche models predict current over-wintering sites in Mexico will be unsuitable for monarchs in 40 to 50 years. Climate is likely to be equally or more important during the summer. Here, I seek to predict the consequences of climate change for monarchs during the summer breeding season and fall migration and determine their ability to cope with these changes.

Using ecological niche modeling, I predicted a marked northward range shift throughout the summer breeding season, necessitating movement before each summer generation and a longer, faster migration. It is unclear whether monarchs can track geographic changes to their ecological niche, and if not, they stand to lose up to half their available breeding habitat.

If monarchs can withstand temperatures hotter than their current ecological niche, their range shifts may not be as drastic. I assessed the lethal and sub-lethal impacts of exposure to temperatures above their predicted ecological niche. Daytime temperatures of 38 and 40°C caused a slight increase in mortality, as well as difficulty pupating, smaller adult size and a longer development time. Daytime temperatures of 42°C caused substantially more mortality and no larvae survived exposure to 44°C. These results suggest that the ecological niche models are accurate and monarchs could lose a substantial amount of available breeding habitat.

To assess the monarch butterfly's potential to cope with a changing climate and adjust its migration, I aimed to identify any level of plasticity currently present in the

migration. An introduced species of milkweed, *Asclepias curassavica*, present in Texas, may be changing the monarchs' breeding habits during its fall migration. Data from the Monarch Larva Monitoring Project and field studies I conducted indicate that female monarchs prefer the introduced milkweed to native milkweed particularly during the fall migration. If monarchs are already changing their migration in response to this environmental change, there may be the variability in monarch migratory and reproductive behavior that could allow this species to adjust to future climate change.

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INTRODUCTION

Several aspects of insect biology make it likely that their distributions will be affected by global climate change. As poikilotherms, their physiology is directly affected by external climatic conditions. Also, many insects are closely tied to host plants and will thus be affected by vegetation shifts that accompany a changing climate (IPCC 1996, Crowley 2000, Peñuelas 2002).

Holt (1990) describes three general expectations for species' responses to climate change: movement, adaptation, or extirpation. For the bulk of sedentary wild species, ranges may take decades or centuries to shift noticeably. However, butterflies, particularly highly mobile or migratory species, may be able to track their ecological niche as the climate changes (Parmesan et al. 1999, Parmesan and Yohe 2003). Non-migratory European butterflies exhibit evidence of a northward range expansion over the last century (Parmesan et al. 1999). In the United States, the northern boundary of *Atalopedes campestris* is now in Washington State, 420 miles north of where it was in California 35 years ago (Crozier 2004). These, and range shifts documented in other taxa, are best explained by anthropogenic climate change (Parmesan and Yohe 2003). Despite the presence of suitable habitat at lower elevations, Edith's checkerspot butterfly (*E. editha*) populations shifted towards higher elevation in concert with changes in winter snow levels (Parmesan 1996, 2003).

In addition to shifts in species' distributions, climate change has already caused changes to phenology. The date of first spring appearance is earlier for 26 of 35 species of butterflies examined in the United Kingdom (Roy and Sparks 2000). In California,

seventy percent of 23 butterflies species advanced the timing of their first flight, with warmer, drier winters driving the change (Forister and Shapiro 2003).

Monarch butterflies (*Danaus plexippus*) are ideal organisms for studying the effects of climate change. These well-known insect migrants are climatically sensitive; their migration and diapause are triggered by changes in temperatures, light levels, and host plant quality associated with changing seasons (Goehring and Oberhauser 2002). Monarchs tolerate some anthropogenic disturbance, because many of their host plants (plants in the genus *Asclepias* (milkweed)) thrive in disturbed areas, such as along roadsides, in ditches, and in agricultural fields (Oberhauser et al. 2001). This is particularly true of *A. syriaca*, the most commonly utilized host plant. Also, the distribution of monarchs is well known, thanks to a wealth of spatial and temporal data that have been collected by citizen monitors and other observers.

Monarch butterflies cannot survive temperate winters, thus the eastern NA population migrates each fall from wide-ranging summer breeding grounds in north-central and north-eastern US and southern Canada to high-altitude forests of Central Mexico (Brower 1995). The same butterflies fly from Mexico in the spring to the southern US where they begin to breed. Their offspring continue northward, perhaps in response to encroaching summer heat and a decrease in milkweed availability. Once in their primary breeding range, there are two or three stationary generations before the fall migration. Monarch populations in other areas exhibit a variety of migratory behaviors; those in Central and South America, the West Indies, and Hawaii do not migrate, while those in western NA and Australia migrate considerably shorter distances than the eastern

NA population (Ackery & Vane-Wright 1984, James 1993, Brower 1995, Altizer and Oberhauser 2000).

Physiological differences exist between and within monarch populations. In the eastern NA population, summer generations of butterflies fly singly, disperse randomly, and reproduce soon after emerging as adults. In contrast, fall migrants are in a state of reproductive diapause, do not search for mates, and possess fat stores to be used during the six-month wintering period (Kammer 1971). Monarchs in Australia, and perhaps California, with a truncated migration, do not go into diapause; rather, they have a shortened period of reproductive dormancy called oligopause (James 1982). Non-migrating populations do not exhibit diapause behavior.

Monarch butterflies are already believed to be vulnerable to the effects of climate change. Oberhauser and Peterson (2003) modeled the effects of projected global climate change on the over-wintering habitats of the eastern NA monarch population. According to their ecological niche models, current over-wintering sites in Mexico will be unsuitable for monarchs in 40 to 50 years. Already, a January 2002 winter storm marked by extreme cold and precipitation caused the most severe winter kill of monarchs known, with 70-80% mortality in two major over-wintering colonies (Brower et al. 2004), and a storm of similar magnitude and effect occurred in 2004. The frequency of such storms is predicted to increase with climate change (Oberhauser and Peterson 2003).

Climate is likely to be equally or more important during the summer breeding season. Extensive rain or prolonged cool and cloudy conditions can reduce egg laying and increase development time, while prolonged hot or dry spells can reduce adult lifespan and fecundity. Zalucki and Rochester (2004) predicted large fluctuations in

monarch abundance in the eastern NA population due to the effects of climate on their phenology and fecundity. Climate can also influence milkweed abundance and quality (Zalucki and Rochester 2004). Milkweed quality deteriorates under high temperatures, making it less attractive to egg-laying females and larvae (Zalucki and Kitching 1982).

Additional research is needed to further elucidate the consequences of climate change for monarch butterflies during their migration and the summer breeding months. Here, I assessed the potential impact of climate change to the geographic location and abundance of monarch habitat. Using ecological niche modeling, I identified the current niche characteristics of breeding monarchs and projected these models onto future climate patterns, predicting necessary range shifts and possible habitat losses (Chapter 1). As ecological niche models are based on occurrence data, I also conducted laboratory experiments to identify lethal and sub-lethal effects of exposures to temperatures warmer than the monarchs' current niche (Chapter 2). If monarchs can withstand temperatures outside of their predicted niche, habitat losses and range shifts predicted by niche models may not be as severe. If monarchs are to cope with the predicted changes, some level of plasticity needs to be present in their movement patterns. There is evidence that monarchs are laying eggs in Texas as they migrate through on route to Mexico. Through field surveys and experiments, I investigate the possible causes of fall breeding, specifically the impact of an introduced species of milkweed (Chapter 3). As the fall breeding monarchs are by definition not in diapause, as they are thought to be during the migration, this may represent variation in the population that could allow the eastern NA population to cope with habitat changes brought on by climate change. All together, this work aims to estimate the potential impacts of climate change on the eastern NA monarch

population and the level of plasticity in their migration and breeding that could allow them to cope with predicted changes.

REFERENCES

- Ackery, P. R. and R. I. Vane-Wright. 1984. *Milkweed butterflies: Their cladistics and biology*. Ithaca: Cornell University Press, Comstock Publishing Associates.
- Altizer, S. M. and K. S. Oberhauser. 2000. Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. *Ecol. Entomol.* 25: 125-39.
- Brower, L. P. 1995. Understanding and misunderstanding the migration of the monarch butterfly (Nymphalidae) in North America: 1857-1995. *J. Lepid. Soc.* 49:304-85.
- Brower, L. P., D. R. Kust, E. Rendon-Salinas, E. G. Serrano, K. R. Kust, J. Miller, C. Fernandez del Ray, and K. Pape. Catastrophic Winter Storm Mortality of Monarch Butterflies in Mexico in January 2002. in Oberhauser, K. S. and M. J. Solensky. 2004. *Monarch Butterfly Biology and Conservation*. Cornell University Press.
- Crowley, T. J. 2000. Causes of climate change over the past 1000 years. *Science* 289: 270-277.
- Crozier, L. G. 2004. Field transplants reveal summer constraints on a butterfly range expansion. *Oecologia*. 141: 148-157.
- Forister M. L., Shapiro A. M. 2003. Climatic trends and advancing spring flight of butterflies in lowland California. *Glob. Change Biol.* 9: 1130–35.
- Goehring, L. and K. S. Oberhauser. 2002. Effects of photoperiod, temperature, and host plant age on induction of reproductive diapause and development time in *Danaus plexippus*. *Ecol. Ent.* 27: 674-685.
- Holt, R. D. 1990. The microevolutionary consequences of climate change. *Trends Ecol. Evol* 5: 311-315.
- Intergovernmental Panel on Climate Change. 1996. in *Climate Change 1995, Impacts, Adaption and Mitigation of Climate Change: Scientific-Technical Analyses*. Cambridge Univ. Press, Cambridge U.K. pp. 171-189.
- James, D. G. 1982. Ovarian dormancy in *Danaus plexippus* (L.) (Lepidoptera Nymphalidae)—oligopause not diapause. *J. Aust. Entomol. Soc.* 21: 31-35.

- James, D.G. 1993. Migration biology of monarchs in Australia. in *Biology and conservation of the monarch butterfly*, eds. Malcolm, S. B. and M. P. Zalucki (Natural History Museum of Los Angeles County, Los Angeles, CA), pp. 189-200.
- Kammer, A. E. 1971. Influence of acclimation temperature on the shivering behavior of the butterfly *Danaus plexippus* (L.). *Zeitschrift Fur Vergleichende Physiologie* 72:364-369.
- Oberhauser, K. S., M. D. Prysby, H. R. Mattila, D. E. Stanley-Horn, M. K. Sears, G. Dively, E. Olson, J. M. Pleasants, W. F. Lam and R. L. Hellmich. 2001. Temporal and spatial overlap between monarch larvae and corn pollen. *Proc. Natl. Acad. Sci.* 98: 11913-11918.
- Oberhauser, K. and A. T. Peterson. 2003. Modeling current and future potential wintering distributions of eastern North American monarch butterflies. *Proc. Natl. Acad. Sci.* 100: 14063-14068.
- Parmesan C. 1996. Climate and species' range. *Nature* 382: 765–66.
- Parmesan, C., N. Ryrholm, C. Stefanescu, J. K. Hill, C. D. Thomas, H. Descimon, B. Huntley, L. Kaila, J. Kullberg, T. Tammaru, et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*. 399: 579-583.
- Parmesan C. 2003. Butterflies as bio-indicators of climate change impacts. In *Evolution and Ecology Taking Flight: Butterflies as Model Systems*, ed. C. L. Boggs, W. B. Watt, P. R. Ehrlich, pp. 541–60. Chicago: Univ. Chicago Press.
- Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37-42.
- Peñuelas, J., I. Filella, and P. Comas. 2002. Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. *Global Change Biology* 8: 531-544.
- Roy D. B., T. H. Sparks. 2000. Phenology of British butterflies and climate change. *Glob. Change Biol.* 6: 407–416.
- Zalucki, M. P. and R. L. Kitching. 1982. Temporal and spatial variation of mortality in field populations of *Danaus plexippus* L. and *D. chrysippus* L. larvae (Lepidoptera: Nymphalidae) *Oecologia* 53: 201-207.
- Zalucki, M. P. and W. A. Rochester. 2004. Spatial and temporal population dynamics of monarchs down-under: lessons for North America, pp. 219-228. In K. Oberhauser, and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.

CHAPTER 1

Ecological Niches in Sequential Generations of Eastern North American Monarch Butterflies (Lepidoptera: Danaidae): The Ecology of Migration and Likely Climate Implications

SUMMARY

Eastern North American monarch butterflies (*Danaus plexippus* L.) show a series of range shifts during their breeding season. Using ecological niche modeling, we studied the environmental context of these shifts by identifying the ecological conditions that monarchs use in successive summer months. Monarchs use a consistent ecological regime through the summer, but these conditions contrast strikingly with those used during the winter. Hence, monarchs exhibit niche-following among sequential breeding generations, but niche-switching between the breeding and overwintering stages of their annual cycle. We projected their breeding ecological niche onto monthly future climate scenarios, which indicated northward shifts, particularly at the northern extreme of their summer movements, over the next 50 years; if both monarchs and their milkweed host plants cannot track these changing climates, monarchs could lose distributional area during critical breeding months.

INTRODUCTION

Monarch butterflies (*Danaus plexippus* L.) are perhaps the best-known of migratory insects. Because they cannot overwinter in temperate climates, the eastern North American population migrates from broad summer breeding grounds in the United

States and Canada to small areas of high-altitude fir forest in central Mexico. The same butterflies fly from Mexico to the southern United States in early spring, where they begin breeding. Their offspring continue northward, perhaps because of increasing heat and humidity and decreased host plant availability (Malcolm et al. 1987, 1993), and a subsequent generation returns to Mexico in the following fall. The close ties between this organism's annual cycle and climatic conditions suggest that monarchs could be impacted negatively by ongoing global climate change processes (Oberhauser and Peterson 2003).

Zalucki and Rochester (2004) predicted large-scale fluctuations in abundance of eastern North American monarchs resulting from effects of climate on phenology and fecundity. Prolonged rainy, cloudy, and cool conditions can reduce egg-laying and increase development time, whereas prolonged hot, dry spells can reduce adult lifespan and fecundity (Zalucki 1981). Extended exposure to temperatures of $\geq 36^{\circ}\text{C}$ can cause significant larval mortality and developmental changes (Zalucki 1982, York and Oberhauser 2002). Climate can also influence the abundance and quality of monarchs' host plant; monarch larvae are specialist herbivores, feeding solely on plants in the genus *Asclepias* (milkweeds), and milkweed quality for developing larvae deteriorates at high temperatures (Zalucki and Kitching 1982).

Here, we assess seasonal variation in ecological niche characteristics of breeding monarch butterfly populations, taking advantage of recent advances in assessing seasonal niche variation (Joseph and Stockwell 2000, Martinez-Meyer et al. 2004, Nakazawa et al. 2004), to understand the constancy of monarchs' ecological requirements through the year. We then investigate likely effects of global climate change on these seasonal

patterns, projecting the present-day predictive models to future (2055) climate patterns to outline future potential distributional areas and migratory shifts. Results are interpreted in the context of the migratory behavior in the eastern North American monarch population, emphasizing the unique nature of the annual migration and high-altitude tropical overwintering behavior of these populations.

METHODS

Input data sets

Primary point-occurrence data for the eastern North American monarch population were obtained from the Monarch Larva Monitoring Project (MLMP). MLMP has recruited a cadre of volunteers to obtain date-specific occurrence data for immature monarchs across 32 U.S. states and 2 Canadian provinces since 1997 (<http://www.mlmp.org>). The MLMP dataset describes spatial and temporal variation in juvenile abundance and survival, as well as in productivity across different habitat types (Pryby and Oberhauser 2004). For this analysis, we only used egg occurrence data; similarly, for simplicity, we did not consider abundances, but rather focused on presences at particular locations and times (Table 1).

Queen butterfly [*Danaus gilippus* Cramer (Lepidoptera: Nymphalidae)] eggs are indistinguishable from monarch eggs, but queen butterfly larvae possess a third set of tentacles not present in monarchs. As the ranges of these two species overlap in the southern United States, some MLMP volunteers may report monarch egg presence when they are actually seeing queen butterfly eggs. Hence, to verify data in the southern United States, we identified instances in which eggs were observed in one week but no monarch

larvae were observed the following week. Without the confirmation of monarch larvae, these observations could be due to the presence of queens. Volunteers at these five sites (all in Texas) were contacted and asked to confirm their data; none reported sightings of queen butterfly larvae near the time in question. Therefore, we assumed the observed eggs were monarchs, and included this data in our models.

Ecological dimensions initially assessed included raster GIS data layers describing 5 climatic and 4 topographic parameters (maximum, minimum, and mean monthly temperatures; monthly solar radiation; monthly precipitation; elevation; slope; aspect; and tendency to pool water). Ecological niches were characterized using climatic variables for the period 1961-1990 (New et al. 1997), and topographic data were obtained from the Hydro-1K dataset (<http://edcdaac.usgs.gov/gtopo30/hydro>). To permit efficient computing, all present-day variables were resampled to 0.1° spatial resolution (~10 km). Model development was limited to the area within 500 km of MLMP sampling points. We excluded occurrence data for the resident population in southern Florida and populations west of the Rocky Mountains, as their migratory habits (and perhaps their ecological requirements) differ from those of the eastern population. We prepared month-specific data sets of monarch occurrences and ecological variables for March through September, as well as for the overwintering period (December-February). We performed a jackknife manipulation, in which single data layers were omitted sequentially and effects on omission error assessed, to identify data layers that did not contribute positively to overall predictive success, following Peterson and Cohoon (1999).

To predict future potential distributions, we used two scenarios of HadCM2, a general circulation model (Carson 1999) that has been used to create scenarios of future

climate conditions. The HHGSDX50 scenario assumes 0.5%/yr CO₂ increase (IS92d), and incorporates mitigating effects of sulphate aerosol forcing, making it a relatively conservative estimate of climate change. The HHGGAX50 scenario assumes a 1%/yr CO₂ increase (IS92a) and does not allow for effects of sulfate aerosols, and so is more extreme. As they are based on a 30 year average around 2055, our models do not take into account potential effects of increased climate variability (El Niño events, in particular) on species' distributions. Because these future climate data are provided at a very coarse spatial resolution of 2.5° × 3.75°, we calculated expected changes in temperature (°C) and precipitation (mm) under each scenario from the relatively coarse raw model results; these expected changes were applied to the original Intergovernmental Panel on Climate Change current climate data layers to provide a final pixel resolution of ~30 × 30 km for future-climate data layers.

Niche models

We modeled month-specific ecological niches using a desktop implementation of the Genetic Algorithm for Rule-set Prediction (GARP) (Stockwell 1992, Stockwell 1999, Stockwell and Peters 1999, <http://www.lifemapper.org/desktopgarp>). GARP is a machine-learning application that seeks non-random associations between point-occurrence data and ecological information in the form of raster GIS data layers in an iterative process of random rule generation, evaluation, perturbation, testing, and incorporation or rejection. The resulting models identify portions of ecological space that reflect the niche dimensions relevant to the geographic distributions of species. It should, of course, be borne in mind that these models are correlative, and that niches almost

certainly vary in ecological dimensions not included in the present study. The approach has been described in detail elsewhere (Soberón and Peterson 2005).

We developed ecological niche models for March through September (the month on which a particular model is based is called the “focal month”) and projected each focal month model onto climatic conditions for each other breeding month, the winter months (December through February) and future climate scenarios for the focal month. We tested the robustness of the model using projections in which the focal month was projected onto itself and used to predict a random 50% of available occurrence points set aside for a relatively independent test of model quality. Projections of the focal month onto each other month indicated whether monarchs used the same ecological niches throughout the summer breeding period. The changed-climate projections allowed us to predict where acceptable breeding conditions might exist under future climates. Details for each of these steps follow.

To optimize ecological niche model quality, we developed 100 replicate models for each month-to-month comparison based on random 50-50 splits of available occurrence points. Half of the occurrence points were used to build models; the others were used to test them. Of these 100 models, we selected the 10 ‘best’ based on error component distributions, (Anderson et al. 2003). This procedure is based on the observations that (1) models vary in quality, (2) variation among models involves an inverse relationship between error of omission (leaving out true distributional area) and commission (including areas not actually inhabited), and (3) best models (as judged by experts blind to error statistics) are clustered in a region of minimum omission of independent test points (obtained from the 50-50 splits) and moderate area predicted

present (an axis related directly to commission error). The position of a particular model in relation to the two error axes provides an assessment of the relative accuracy of each model. To choose best subsets of models, we (1) eliminated all models that had >5% omission error based on independent test points, (2) calculated the median area predicted present among these low-omission models, and (3) identified models within the central 50% of the overall frequency distribution of area predicted present.

Ecological similarity among monthly ecological niche models was assessed through interpredictivity calculations (Peterson et al. 1999, Peterson and Vieglais 2001, Martínez-Meyer 2002), which are based on the premise that if two populations share similar ecological niches, then the models for one will predict the distribution of the other accurately, and vice versa. This approach involved projection of models for the focal month onto the environmental data sets for each other month, and overlay of occurrence data for the other months. Niche similarity was measured as the percentage of occurrence data points falling within the prediction area of the model (Peterson and Vieglais 2001), reported in a matrix, in which cell values summarize the percentage of occurrences predicted correctly by one month's model in each other month studied. To assess statistical significance, we compared observed predictive success with the expected based on random association between predictions and test points: the proportion of the study area predicted by the model to be present or absent was multiplied by number of test points that yielded random expectations (Peterson 2001, Peterson and Vieglais 2001). Observed and expected numbers were compared using a one-tailed chi-squared test, with 1 degree of freedom (calculated in Microsoft Excel). (It should be noted that, lacking data

on absences of the species across the landscape, the more customary kappa and ROC AUC statistics are not applicable.)

Future potential monthly distributional areas were estimated through methods described in detail elsewhere (Araujo et al. 2005, Peterson et al. 2001, Peterson et al. 2002, Peterson 2003, Thomas et al. 2004). In brief, we projected the present-day, month-specific ecological niche models onto future month-specific climate data, and averaged the projections from the two future-climate scenarios. We summarized climate change effects as (1) raw area predicted present before climate change, (2) raw area predicted present after climate change, and (3) area of overlap between (1) and (2). (2) is equivalent to a ‘universal dispersal’ assumption, whereas (3) is equivalent to a ‘no dispersal’ assumption (Thomas et al. 2004).

RESULTS

Initial jackknife manipulations of the climatic and topographic parameters found that aspect, solar radiation and tendency to pool water did not contribute significantly to model quality, so our models of present-day distributions were based on maximum, minimum, and mean monthly temperatures; precipitation; elevation; and slope. This suite of variables allowed highly accurate month-specific models, as assessed in predicting independent test data sets (chi-squared values ranged from 4.2 to 211.9 for all 7 monthly chi-squared tests, $df = 1$, all $P < 0.05$).

Predictivity among monthly ecological and geographic distributions was excellent (Figure 1); coincidence between predictions and relatively independent test points was considerably better than random expectations when models for focal months were used to

predict occurrence data from each other month (Table 2). Models for April, May, and June predicted monarch distributions for every month significantly better than random models. The July ecological niche model predicted independent test data sets well for May through August, and August predicted April through August. March and September were more erratic in their ability to predict other months, likely reflecting lower sample sizes available for model-building and the fact that temperatures are more variable in those two months. Hence, monarchs appear to be faithful ‘niche followers’ throughout their breeding period.

Comparing ecological niches as modeled for the breeding (herein) and overwintering stages (Oberhauser and Peterson 2003), however, reveals a niche shift between the two seasons. The breeding season niche is characterized by warmer and wetter conditions than monarchs occupy during the winter (Figure 2); this difference is reflected in poor (no) coincidence between predictions from models based on breeding sites and overwintering points. During the winter months, breeding niche conditions are manifested in Atlantic coastal portions of Central America, as opposed to the central Mexican highlands where monarchs overwinter (Oberhauser and Peterson 2003).

Projecting month-specific ecological niche models onto future climate scenarios suggests complex effects of climate change on the seasonal potential geography of monarchs (Figure 3). Early in the breeding season, monarchs could see an increase in the area of ecologically suitable habitat, assuming that they can migrate more quickly and somewhat longer distances. In June-August, however, ideal conditions shift northward and separate more from the current range. The amount of suitable area available in the present and future, based on different assumptions of dispersal ability (universal dispersal

or no dispersal assumption) is shown in Figure 4. This illustrates the complexities of likely climate change effects on monarch seasonal geography.

DISCUSSION

Recent years have seen many applications of ecological niche modeling approaches to questions of distributional biology of species. Applications have included describing distributions, discovering new populations and species, describing historical distributional shifts, predicting the geographic potential of species' invasions, and anticipating future distributional shifts in the face of changing conditions (Zalucki and Rochester 1999, Guisan and Hofer 2003, Pearson and Dawson 2003, Thuiller et al. 2005, Wiens and Graham 2005, Araújo and Guisan 2006, Araújo et al. 2006, Pearson et al. 2006, Thuiller et al. 2006). Although methodologies and thought frameworks are in the process of rapid development, applications to seasonal biology are still few (Martínez-Meyer et al. 2004, Nakazawa et al. 2004, Peterson et al. 2005). As such, this review of seasonal ecology of monarchs represents an important addition to a growing literature.

The ability of ecological niche models to predict month-to-month distributional dynamics demonstrates that sequential generations of breeding monarchs are effectively 'niche followers' across the changing ecological landscape of spring, summer and fall. Some focal months were better able to predict seasonal shifts than others; causes may include the smaller occurrence data sets available in March and September (Table 1), the fact that monarchs are switching from migratory to breeding behavior, and the accelerated rate at which seasonal climates change during these months. Because MLMP volunteers do not always record absence data, input data from the beginning and end of

the breeding season may be less reliable. More generally, our monthly resolution may provide a temporal scale too coarse for precise predictions, particularly in spring and fall, when climates are changing more rapidly.

The lack of correspondence between the breeding season niche and the winter niche used by monarch butterflies is evidence that monarchs switch niches as they migrate to and from their wintering sites in central Mexico, even though their breeding niche is available in other (albeit more distant) parts of Mexico and Central America during the winter months. This result effectively combines the ‘niche follower’ and ‘niche switcher’ dichotomy of Nakazawa et al. (2004) within a single species—monarchs follow a breeding-season niche from March-October, but spend the remainder of the year under very different conditions.

Future-climate projections of month-specific ecological niche models predict marked changes in the species’ seasonal potential distribution. Currently, monarchs migrate from Mexican wintering grounds to breed in the southern United States, and their offspring migrate into more northern areas. The current spatial manifestation of the niche shifts northward during March-May, but remains relatively stationary in June-August (Figure 1). With climate change, habitable areas are predicted to continue shifting northward through July (Figure 3). The seasonal potential distribution then contracts in August, perhaps necessitating relocation of all local populations of monarchs before breeding. It is unclear whether monarchs will be able to adjust their seasonal movement patterns to accommodate these changing conditions or what the consequence of lost breeding locations will be.

Mobile species such as monarchs may be able to track the geographic distribution of their breeding ecological niches seasonally as climates changes; evidence indicates that some European butterflies have achieved such tracking (Parmesan et al. 1999, Hill et al. 2003). Additionally, monarch diapause behavior and movement patterns have changed as the species has expanded into new habitats (e.g., Zalucki and Rochester 1999). If monarchs can indeed track the shifts in ecological niche conditions (universal dispersal assumption), they stand to gain a significant amount of habitat (Figure 4). Currently, the range of milkweed extends only 160 km into Canada, so it is absent from the regions of Canada to which monarchs' potential distributions are projected to shift (Woodson 1954). Milkweed may not be able to colonize northward as rapidly as monarchs might require, which may make these habitats nonetheless uninhabitable. Without such universal dispersal potential (no dispersal assumption), monarchs could lose considerable potential distributional area in June-August (Figure 4), the most critical breeding portion of the annual cycle.¹

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REFERENCES

- Anderson, R. P., D. Lew, and A. T. Peterson. 2003. Evaluating predictive models of species distributions: criteria for selecting optimal models. *Ecol. Mod.* 162: 211-232.
- Araújo, M. B., R. G. Pearson, W. Thuiller, and M. Erhard. 2005. Validation of species-climate impact models under climate change. *Glob. Change Biol.* 11: 1504-1513.
- Araújo, M. B., and A. Guisan. 2006. Five (or so) challenges for species distribution modelling. *J. Biogeogr.* 33: 1677-1688.
- Araújo, M. B., W. Thuiller, and R. G. Pearson. 2006. Climate warming and the decline of amphibians and reptiles in Europe. *J. Biogeogr.* 33: 1712-1728.
- Carson, D. J. 1999. Climate modelling: achievements and prospects. *QJR Meteorol. Soc.* 125: 1-27.
- Guisan, A., and U. Hofer. 2003. Predicting reptile distributions at the mesoscale: relation to climate and topography. *J. Biogeogr.* 30: 1233-1243.
- Hill, J. K., C. D. Thomas, and B. Huntley. 2003. Modelling present and potential future ranges of European butterflies using climate response surfaces, pp. 149-167. In C. L. Boggs, W. B. Watt, and P. R. Ehrlich (eds.), *Butterflies Ecology and Evolution Taking Flight*. University of Chicago Press, Chicago, IL.
- Joseph, L. and D. R. B. Stockwell. 2000. Temperature based models of the migration of Swainson's flycatcher *Myiarchus swainsoni* across South America: a new use for museum specimens of migratory birds. *Proc. Acad. Nat. Sci. Philad.* 150: 293-300.
- Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1987. Monarch butterfly voltinism: effects of temperature constraints at different latitudes. *Oikos.* 49: 77-82.
- Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1993. Spring recolonization of eastern North America by the monarch butterfly: successive brood or single sweep migration?, pp. 253-267. In S. B. Malcolm and M. P. Zalucki (eds.), *Biology and Conservation of the Monarch Butterfly*. Publications of the Los Angeles County Museum of Natural History, Los Angeles, CA.
- Martínez-Meyer, E. 2002. Evolutionary Trends in Ecological Niches of Species. Ph.D. dissertation, University of Kansas, Lawrence.
- Martínez-Meyer, E., A. T. Peterson, and A. G. Navarro-Sigüenza. 2004. Evolution of seasonal ecological niches in the Passerina buntings (Aves: Cardinalidae). *Proc. R. Soc. Lond. B.* 271: 1151-1157.

- Nakazawa, Y., A. T. Peterson, E. Martínez-Meyer, and A. G. Navarro-Sigüenza. 2004. Seasonal niches of neararctic-neotropical migratory birds: implications for the evolution of migration. *Auk*. 121: 610-618.
- New, M., M. Hulme and P. Jones. 1997. A 1961-1990 Mean Monthly Climatology of Global Land Areas. Norwich, U.K. Climatic Research Unit, University of East Anglia.
- Oberhauser, K. and A. T. Peterson. 2003. Modeling current and future potential wintering distributions of eastern North American monarch butterflies. *Proc. Natl. Acad. Sci.* 100:14063-14068.
- Parmesan, C., N. Ryrholm, C. Stefanescu, J. K. Hill, C. D. Thomas, H. Descimon, B. Huntley, L. Kaila, J. Kullberg, T. Tammaru, et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*. 399: 579-583.
- Pearson, R. G., and T. P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecol. Biogeogr.* 12: 361-371.
- Pearson, R. G., C. Raxworthy, M. Nakamura, and A. T. Peterson. 2006. Predicting species' distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *J. Biogeogr.* 34: 102-117.
- Peterson, A.T. and K. C. Cohoon. 1999. Sensitivity of distributional prediction algorithms to geographic data completeness. *Ecol. Mod.* 117: 159-164.
- Peterson, A. T., J. Soberon, and V. Sanchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. *Science*. 285: 1265-1267.
- Peterson, A. T. 2001. Predicting species' geographic distributions based on ecological niche modeling. *Condor*. 103: 599-605.
- Peterson, A. T., V. Sánchez-Cordero, J. Soberón, J. Bartley, R. H. Buddemeier and A. G. Navarro-Sigüenza. 2001. Effects of global climate change on geographic distributions of Mexican Cracidae. *Ecol. Mod.* 144: 21-30.
- Peterson, A. T. and D. A. Vieglais. 2001. Predicting species invasions using ecological niche modeling: new approaches from bioinformatics attack a pressing problem. *BioScience* 51: 363-371.
- Peterson, A. T., M. A. Ortega-Huerta, J. Bartley, V. Sánchez-Cordero, J. Soberón, R. H. Buddemeier, D. R. B. Stockwell. 2002. Future projections for Mexican faunas under global climate change scenarios. *Nature*. 416: 626-629.

- Peterson, A. T. 2003. Projected climate change effects on Rocky Mountain and Great Plains birds: generalities of biodiversity consequences. *Glob. Change Biol.* 9: 647-655.
- Peterson, A. T., C. Martínez-Campos, Y. Nakazawa, and E. Martínez-Meyer. 2005. Time-specific ecological niche modeling predicts spatial dynamics of vector insects and human dengue cases. *Trans. R. Soc. Trop. Med. Hyg.* 99: 647-655.
- Prysbý, M. D. and K. Oberhauser. 2004. Temporal and Geographic Variation in Monarch Densities: citizen scientists document monarch population patterns, pp. 9-20. In K. S. Oberhauser and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.
- Soberón, J. and A. T. Peterson. 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. *Biodiversity Informatics*. 2: 1-10.
- Stockwell, D. R. B. 1992. Machine learning and the problem of prediction and explanation in ecological modeling. Ph.D. dissertation, Australian National University, Canberra, Australia.
- Stockwell, D. R. B. 1999. Genetic algorithms II, pp. 123-144. in A. H. Fielding (ed.), *Machine learning methods for ecological applications*. Bluer Academic Publishers, Boston, MA.
- Stockwell, D. R. B. and D. P. Peters. 1999. The GARP modeling system: problems and solutions to automated spatial prediction. *Int. J. Geog. Inform. Sys.* 13: 143-158.
- Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. Ferreira de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. Van Jaarsveld, G. E. Midgely, L. Miles, M. A. Ortega-Huerta, A. T. Peterson, O. L. Phillips, and S. E. Williams. 2004. Extinction risk from climate change. *Nature*. 427: 145-148.
- Thuiller, W., D. M. Richardson, P. Pysek, G. F. Midgely, G. O. Hughes, and M. Rouget. 2005. Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. *Glob. Change Biol.* 11: 2234-2250.
- Thuiller, W., G. F. Midgely, G. O. Hughes, B. Bomhard, G. Drew, M. C. Rutherford, and F. I. Woodward. 2006. Endemic species and ecosystem sensitivity to climate change in Namibia. *Glob. Change Biol.* 12: 759-776.
- Wiens, J. J. and C. H. Graham. 2005 Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu. Rev. Ecol. Syst.* 36: 519-539.
- Woodson, R. E. 1954. The North American species of *Asclepias*. *Ann. Mo. Bot. Gard.*

- York, H. A. and K. S. Oberhauser. 2002. Effects of duration and timing of heat stress on Monarch Butterfly (*Danaus plexippus*) (Lepidoptera: Nymphalidae) development. *J. Kans. Entomol. Soc.* 75: 290-298.
- Zalucki, M. P. 1981. The effects of age and weather on egg laying in *Danaus plexippus* L. (Lepidoptera: Danaidae). *Res. Popul. Ecol.* 23: 318-327.
- Zalucki, M. P. 1982. Temperature and rate of development in *Danaus plexippus* L. and *D. chrysippus* L. (Lepidoptera: Nymphalidae) *J. Aust. Entomol. Soc.* 21: 241-46.
- Zalucki, M. P. and R. L. Kitching. 1982. Temporal and spatial variation of mortality in field populations of *Danaus plexippus* L. and *D. chrysippus* L. larvae (Lepidoptera: Nymphalidae) *Oecologia* 53: 201-207.
- Zalucki, M. P. and W. A. Rochester. 1999. Estimating the effect of climate on the distribution and abundance of the monarch butterfly, *Danaus plexippus* (L.): a tale of two continents, pp. 151-163. In J. Hoth,, L. Merino, K. Oberhauser, I. Pisanty, S. Price, and T. Wilkinson (eds.), *The 1997 North American Conference on the Monarch Butterfly*. Commission for Environmental Cooperation, Montreal, Canada.
- Zalucki, M. P. and W. A. Rochester. 2004. Spatial and temporal population dynamics of monarchs down-under: lessons for North America, pp. 219-228. In K. Oberhauser, and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.

Table 1.1: Monarch Larval Monitoring Program data records in which one or more monarch eggs were observed, and on which ecological niche models were based. Number of U.S. States indicates the approximate geographic extent of the monitoring data for that particular month.

Month	Occurrences with eggs present	Number of monitoring events	Number of U.S. States
March	24	49	2
April	47	125	6
May	130	318	18
June	595	1010	23
July	857	1335	24
August	677	1223	25
September	98	411	19

Table 1.2: Summary of interpredictivity tests among month-specific ecological characteristics and geographic distributions of monarch butterflies. Columns are months used to generate predictions (focal months); rows are months predicted. Cell contents are percent correct prediction of independent test data by any of the 10 best-subsets models; asterisks indicate predictions significantly ($P < 0.05$) better than random models (chi-squared tests, $df = 1$).

Month predicted	Month predicting						
	March	April	May	June	July	August	September
March	100*	100*	70*	40*	0	0	60*
April	100*	94*	94*	88*	19	81*	0
May	49	91*	100*	72*	54*	70*	47*
June	54	100*	96*	100*	89*	86*	0
July	65	100*	94*	96*	100*	79*	17*
August	89	100*	93*	96*	80*	100*	76*
September	97*	89*	91*	83*	0	46	100*

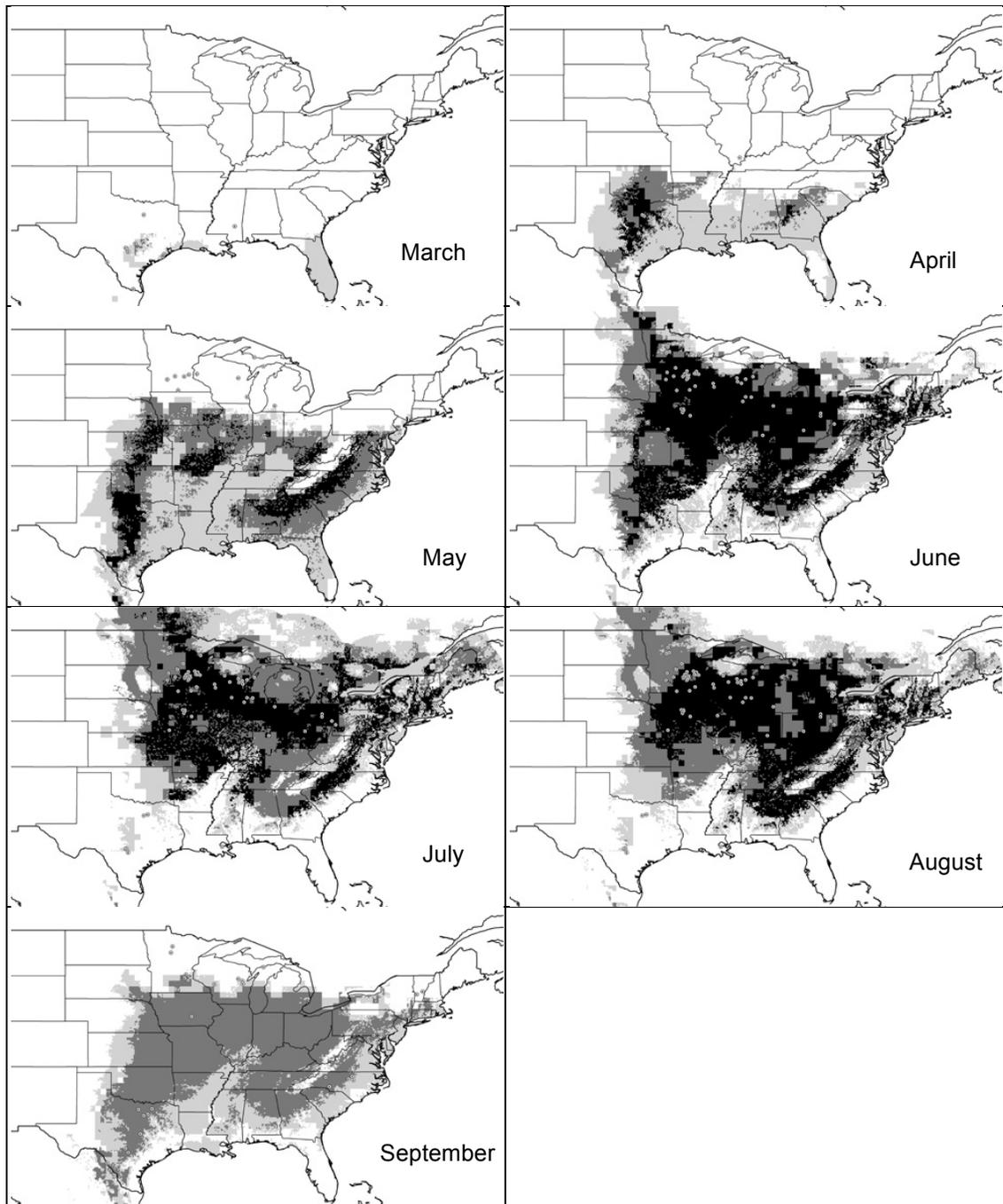


Fig. 1.1: Example of predictivity among monthly ecological and geographic distributions of monarch butterflies: the ecological niche model based on June points used to predict the geographic distribution of the species in March-September. Occurrence data from the month being predicted are overlaid as dotted circles. Predictions are summarized as light

gray = any of 10 best subsets models predicts presence, dark gray = ≥ 6 of 10 best subsets models predict presence, and black = all 10 best subsets models predict presence.

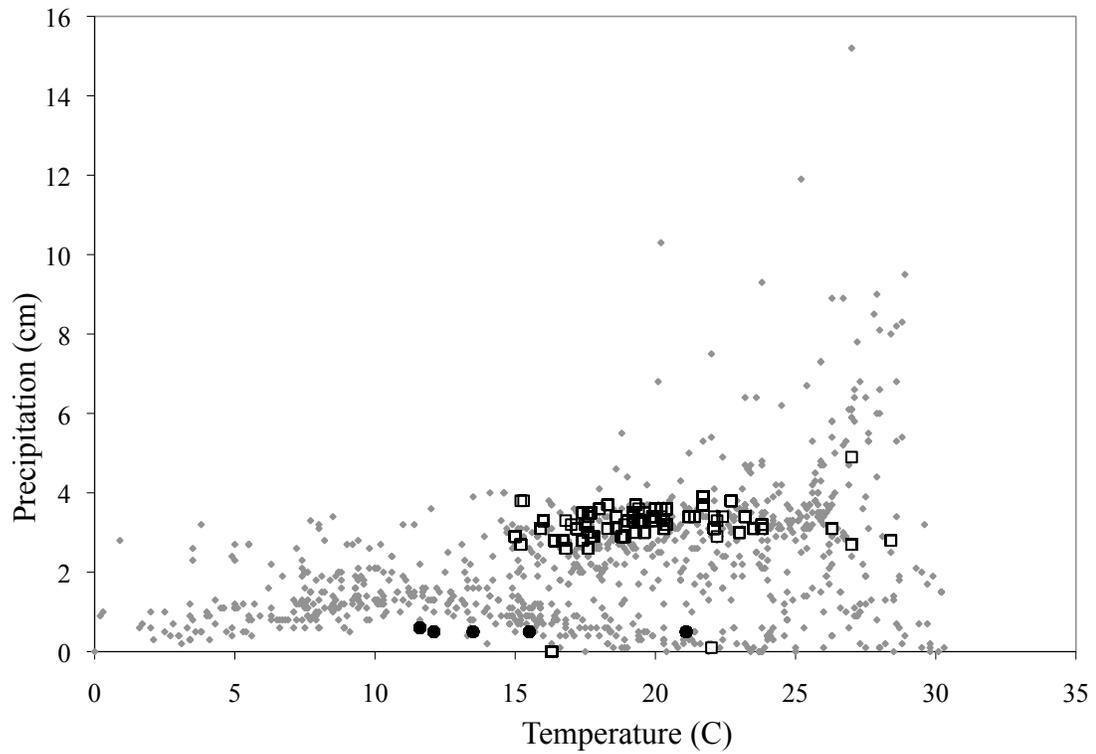


Fig. 1.2: Temperature and precipitation combinations across North America (small gray points, based on June conditions), showing sets of conditions predicted to be suitable for monarchs based on ecological niche models based on June occurrence data (white squares) and based on winter occurrence data of the Mexican overwintering populations (black circles; from Oberhauser and Peterson 2003).

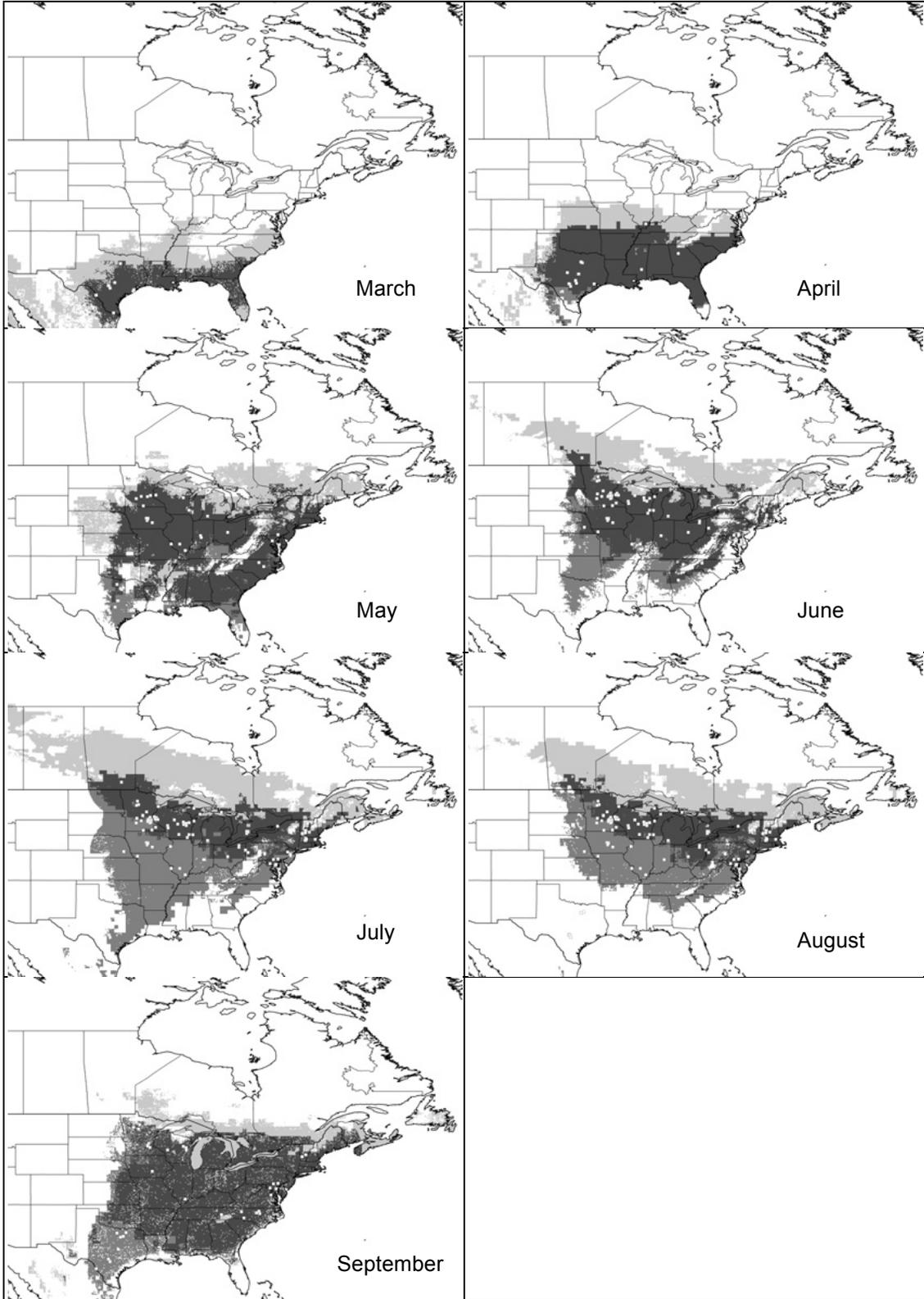


Fig. 1.3: Changed climate (2055) projections for monthly monarch butterfly potential distributions. The present-day distribution is shown in medium gray, and the potential future distribution is shown in light gray; areas of overlap between the two are shown in dark gray. Occurrence points (present day) are plotted as white squares. The maps can be interpreted as follows: medium gray + dark gray = present distribution, light gray + dark gray = future distribution (universal dispersal assumption), and dark gray = future distribution (no dispersal assumption).

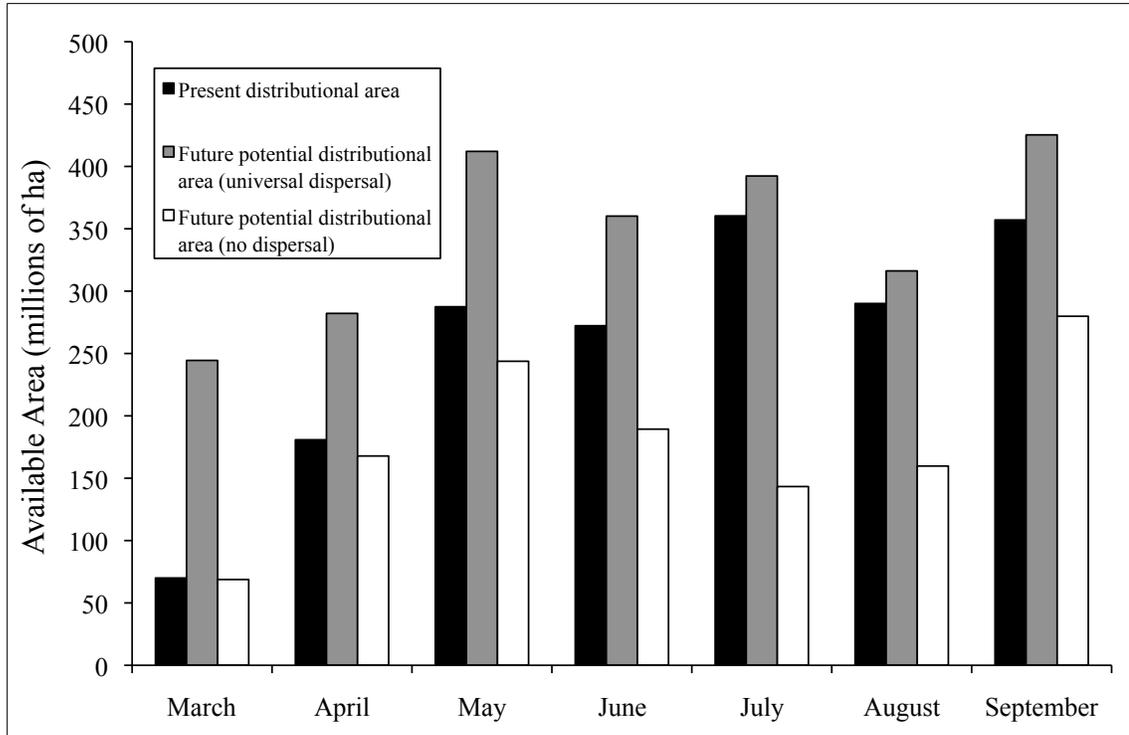


Fig. 1.4: Summary of area estimates for present and future monthly distributional areas for monarch butterflies. Present-day distributional area is shown in black. Projected future potential distributional areas are shown in gray (universal dispersal assumption) and white (no dispersal assumption).

CHAPTER 2

Lethal and Sublethal Effects of Heat Stress on Monarch Butterfly Larvae

SUMMARY

Ecological niche models predict a marked northward summer range shift for eastern North American monarchs, necessitating a longer and faster migration unless monarch larvae can withstand temperatures that are hotter than those in their current habitat. Here, we assess the lethal and sub-lethal effects of exposure to temperatures between 38 and 44°C at the first, third or fifth stadia for 1, 2, 4 or 6 days. Daytime temperatures of 38 and 40°C caused a slight increase in mortality, as well as difficulty pupating, smaller adult size and a longer development time. Daytime temperatures of 42°C caused substantially more mortality and no larvae survived exposure to 44°C. We also tested elevated nighttime temperatures of 30, 32 and 34°C. Mortality and development time increased with increasing duration of exposure. Increased daytime and nighttime temperatures cause higher mortality and negative sub-lethal effects suggesting that monarchs may not be able to cope with warmer temperatures associated with the onset of climate change.

INTRODUCTION

Monarch butterflies (*Danaus plexippus*) are well known insect migrants, with the eastern North American population migrating up to 4500 km from as far north as southern Canada to mountains in central Mexico each fall. They spend the winter

roosting in oyamel fir trees and begin to fly north in late February. En route, females lay eggs in northern Mexico and the southern United States. The second generation leaves the southern United States and spreads across much of the United States east of the Rocky Mountains. There, two to three generations develop before they migrate again. Monarchs are believed to be vulnerable to the effects of climate change in their overwintering sites (Oberhauser and Peterson 2003, Zalucki and Rochester 2004), and during the summer breeding season (Batalden et al. 2007), when extensive rain or prolonged cool and cloudy conditions can reduce egg laying and increase development time, and prolonged hot or dry spells can reduce adult lifespan and fecundity (Zalucki 1981, Masters et al. 1988, Masters 1993). Zalucki and Rochester (2004) predicted large fluctuations in monarch abundance in the eastern North American population due to the effects of climate on their phenology and fecundity. Climate can also influence milkweed abundance and quality (Zalucki and Rochester 2004); for example, milkweed deteriorates under high temperatures, making it less attractive to egg-laying females and larvae (Zalucki and Kitching 1982).

Ecological niche models based on occurrence data obtained from the Monarch Larva Monitoring Project (MLMP, Prysby and Oberhauser 2004) predict a marked northward summer range shift for eastern North American monarchs, necessitating a longer and faster migration (Batalden et al. 2007). MLMP data do not show monarch presence above a monthly mean temperature of 30^oC. Previous studies show that continued exposure to temperatures of 36^oC cause significant larval mortality, but single or repeated 12-hour pulses of this extreme temperature cause development time to increase without increasing mortality (Zalucki 1982, York and Oberhauser 2002). This

indicates the importance of low nighttime temperatures, yet observed climate change and future predictions indicate increased frequency of higher nighttime lows (IPCC 2007). To our knowledge, no studies have examined the lethal and sub-lethal impacts of temperatures above 36^oC or explored the effects of elevated nighttime temperatures on monarch butterflies.

To cope with exposure to elevated temperatures, organisms synthesize heat shock proteins (HSP) (Lindquist 1986). HSP are highly conserved proteins that serve as molecular chaperones (Hartl 1996). Molecular chaperones recognize and bind to other proteins as they are synthesizing and prevent misfolding due to high temperatures. Much of what is known about HSP is due to lab experiments; in nature, movement and other behaviors may enable organisms to avoid heat stress (Huey 1991).

There are three general expectations for species' responses to climate change: movement, adaptation, or extirpation (Holt 1990). Mobile species, such as monarchs, may be able to track their ecological niches as the climate changes; there is evidence that some European and Californian butterflies have done this (Parmesan et al. 1999, Parmesan and Yohe 2003, Crozier 2004). According to ecological niche models, monarchs will need to move northward prior to breeding in June and July, and south in August (Batalden et. al. 2007). Currently, only the spring generation moves before breeding; during the prime breeding months monarchs appear to remain in the same geographic range (MLMP 2011). It is unclear whether the summer generations will respond to poor conditions with movement. If, under a changed climate, an individual monarch survives a stressful temperature regime as a larva, that adult might enjoy higher fitness by relocating, perhaps moving north before breeding to ensure better offspring

survival. This occurs in the spring generation, but the mechanisms that prompt this migration are unclear. If monarchs are to track their moving niche, they will need to respond to different cues than those that signal their spring or fall migration (Goehring and Oberhauser 2002, 2004).

Here, we assess the lethal and sub-lethal effects of exposure to temperatures warmer than the monarchs' current ecological niche (experiment 1). We also test the effects of increased nighttime temperatures (experiment 2).

METHODS

In a series of two experiments, we investigated the lethal and sub-lethal impacts of elevated temperatures on monarch survival and development. Experiment 1 focused on daytime temperatures with the goal of determining the physiological limits of monarch larvae development. Experiment 2 combined a tolerable daytime temperature with increased nighttime temperatures. Tolerable was defined as no increased mortality compared with control treatments, but an increase in sub-lethal impacts, determined in experiment 1.

For both experiments, larvae were raised in Percival growth chambers to control thermoperiod and photoperiod with a 12:12 hour thermophase:cryophase cycle schedule. In experiment 1, individuals were kept in separate 500 ml deli containers with ventilation holes in the lids throughout their development. In experiment 2, they were kept in petri dishes through the fourth stadium, then moved to the deli containers to pupate. In all experiments, control and non-treatment conditions were 30°C during the day and 25°C at

night, with thermoperiod coinciding with photoperiod. Rearing tubs were cleaned and larvae given fresh wild-collected milkweed (*Asclepias syriaca*) daily.

Experiment 1

Experiment 1 was completed in two sequential rounds due to space limitations: round 1 temperatures were 38°C and 40°C, and round 2, 42°C and 44°C. All experimental larvae were offspring of wild-caught individuals, collected from the first generation of monarchs in St. Paul, MN in June 2007.

Within hours of hatching from their eggs, individuals were placed in their rearing containers and randomly assigned an experimental group. The experiment was a full factorial design plus a comparison to the control conditions outlined above. We exposed larvae, beginning at stadium 1, 3 or 5, to 12-hour pulses of 38°C, 40°C, 42°C, or 44°C over periods of 1, 2, 4, or 6 days. Sample sizes ranged from 17 to 20 for each treatment group, including the control.

We checked each container daily to clean and feed larvae, tracking mortality and development. Development time was measured in days and degree days (using 12°C as the threshold temperature) (Zalucki 1982). We assessed two additional sub-lethal impacts of heat exposure: the ability to pupate without falling, and adult size. If individuals fell from the lids of their containers when they attempted to pupate, we used thread and tape to affix the cremaster back to the lid; thus falling during pupation, which would usually be lethal in the wild, was not in our experiment. Adult size was measured as mass and wing length. We measured each individual's mass the same day it eclosed, after the wings dried. The right forewing length measurement is the distance from the base of the wing to the apex.

Experiment 2

Experiment 2 was completed in three sequential rounds, due to space limitations. Each round tested a different treatment temperature, which differed from control during the day and night, rather than just day as in experiment 1. Daytime treatment temperature was 38°C for all replications and 30°C, 32°C or 34°C at night in rounds 1, 2, and 3, respectively. We used the same factorial design for treatment timing and duration as in experiment 1.

Larvae for round 1 were offspring of wild-caught individuals, collected from the first generation of monarchs in St. Paul, MN and western Wisconsin in June 2008. Rounds 2 and 3 individuals were offspring of first-generation lab-reared butterflies. We measured the same lethal and sub-lethal indicators of heat stress as experiment 1.

We used logistic regression to test the effects of temperature, and timing and duration of treatment on survival to adulthood. All factors were treated as categorical variables, and model results are reported in logit scale. While every experiment included a control group that was not exposed to extreme temperatures, the regression analyses did not include the control groups, since their treatments included no values for the timing and duration of treatment. When appropriate, we used Fisher's exact test to compare survival in the control group to other groups, and ANOVA models to compare the continuous values of mass and winglength. All analyses were conducted in JMP v. 5.1.2.

RESULTS

Experiment 1

All monarchs exposed to 44°C died before adulthood and of 234 individuals exposed to 42°C, only 16 survived to be adults (Figure 1c). Therefore, we were unable to compare sub-lethal effects across treatments for these temperature regimes. Mortality did not differ between control groups in rounds 1 and 2 (Fisher's exact test, $p = 1.00$).

The logistic regression model shows two-way interactions between temperature and timing of treatment, and between timing and duration (Table 1). Individuals exposed in the third stadium were less likely to survive to adulthood, particularly after 6 days of exposure, with 70 percent surviving at 38°C and 20 percent surviving at 40°C (Figure 1 and Table 1). At 40°C, there was also an increase in mortality at the fifth stadium after 6 days of exposure with 53 percent surviving to adults. Across timing and duration values, mortality increased for individuals exposed to 42°C, with 6.7 percent survival compared to 82 and 72 percent in the 38°C and 40°C groups respectively.

Out of 451 individuals that survived to pupate, 143 dropped from the lids of their containers during or just after pupation. Under natural conditions, falling is likely to result in death, either directly from the fall or from another source, such as predation. Here, the distance to the floor of the container was small, and when we taped pupae by their cremasters back onto the container lid, subsequent survival was 94 percent. Consequently, this potentially lethal impact of exposure to heat stress was not accurately represented in mortality estimates.

The proportion of individuals that fell during pupation did not depend on temperature, but a logistic regression shows an interaction between the timing and

duration of treatment (Table 2). In particular, individuals exposed at the fifth stadium were more likely to fall at durations of 4 or 6 days, with 58 and 72 percent falling, respectively. If exposed for 1 or 2 days, the likelihood of falling was no more than control (Figure 2). We determined if the effect of longer exposure to heat stress was due to the fact that some larvae pupated while they were still in the heat treatment; a Pearson's Chi-Squared test showed no difference in pupation ability whether the individual pupated in the treatment or after returning to control conditions ($\chi^2=0.218$, d.f. = 1, 63, $p = 0.64$). Individuals exposed during the third stadium also showed elevated risk of falling, with 33, 38 and 46 percent falling if exposed for 1, 2 or 4 days, respectively.

With increasing temperature, male and female mass decreased, and monarchs exposed to temperatures above 38 were smaller than controls (Table 3). However, wing length in the treatment groups did not differ from controls (Table 3). The duration and timing of treatment did not affect adult size.

Development time to adult, measured in days and degree days, was higher in nearly all treatments compared to control (Table 4 and Figure 3a, 3b; only results of the analyses for degree days are shown). Within treatments, the number of days and degree days increased with increasing temperature and duration, and decreased when exposure began at the fifth stadium (Table 4). At 38°C, there was a jump in the number of degree days required when exposure increased from 2 to 4 days during the first and third stadia, whereas at 40°C, there was a more gradual increase in development time with exposure duration (Figure 3a, 3b).

With exposure to elevated temperatures, individuals took more degree days to develop, if we assume that development rates continue to increase with increasing

temperature. However, insects have developmental maxima as well as minima. Thus, we recalculated degree days to exclude time exposed to elevated daytime temperatures, essentially assuming that development ceased at 38 and 40°C. The degree day totals reported in figures 3c and 3d and the central columns of Table 4 reflect all of the time spent in control conditions, but only the 12 nighttime hours each day spent under treatment conditions at 25°C. In the 38°C treatment, individuals in the first and third stadia exposed for 6 days and in the fifth stadium exposed for 4 or 6 days, spent fewer degree days to develop than the control, suggesting that they continued to develop at this temperature but only after prolonged exposure. When exposed to 40°C, only fifth instars exposed for 4 or 6 days used significantly fewer adjusted degree days to develop than control, suggesting that larvae were less able to develop during exposure at 40°C (Figure 3c, 3d, Table 4).

To further understand how the larvae develop under extreme heat, we tested additional models of adjusted degree days. Rather than exclude time spent at treatment temperatures completely, we recalculated degree day totals using values between 18 and 26°C in place of 38 or 40°C, but all other temperature values for nighttime or control treatments remained the same. When treatment temperatures are treated as 22°C, individuals in the 38°C group use the same number of degree days to develop as the control group (Figure 3e). Individuals in the 40°C group exposed as first instars for 6 days or as third instars for 4 days use significantly more degree days to develop than control (Figure 3f). None of the adjusted degree day models we examined resulted in degree day values for individuals in the 40°C group equal to the control group.

We determined when developmental lags occurred by tracking development time by stadia. Individuals exposed during the first stadium, regardless of duration, took longer to molt into the second stadium than control larvae (Table 5). At 38°C, regardless of duration, the average development time in the second stadium was not significantly different from control, even though individuals in the 4 or 6 days of exposure groups were still exposed to elevated temperatures. At 40°C, the average development time during the second stadium was longer than the control only for larvae exposed for 6 days beginning in the first stadium. When individuals exposed during the first stadium reached the third stadium, their development was largely on pace with the control treatment.

Exposure to 38°C during the third stadium did not slow development into the fourth stadium, but at those exposed to 40°C took longer than control individuals to molt into the fourth stadium (Table 5). Development time through the fourth stadium was not significantly different from control, except for those exposed to 40°C for 4 and possibly 6 days.

For individuals entering the elevated temperature regimes at the fifth stadium, 38°C did not slow their development, but at 40°C time during the fifth stadium was higher than for the control group (Table 5). Only individuals exposed for 6 days, regardless of temperature, showed longer development time through the pupa stage.

Sample sizes are low in the 42°C treatment group due to high mortality once the treatment started, but there were both immediate and long-term effects of the heat treatment on development for the larvae that survived the treatment (Table 5).

Experiment 2

Control mortality did not differ among rounds 1, 2 and 3, so our analysis includes comparisons across all rounds (Pearson Chi-square = 1.875, d.f. = 2, 57, $p = 0.39$).

Control development time, however, was different across rounds, so we cannot compare this response variable between rounds ($R^2 = 0.14$, F ratio = 3.61, d.f. = 2, 45, $p = 0.04$).

Round 1 was conducted in mid-summer, while rounds 2 and 3 occurred into late fall, thus milkweed quality may have contributed to the observed changes in development time.

We used a logistic regression to test the effects of temperature, timing and duration of treatment on survival to adulthood. As in experiment 1, all factors were treated as categorical variables. The resulting model shows a two-way interaction between temperature and timing of exposure, and an effect of duration (Table 6). At nighttime temperatures of 34°C, a particularly high level of mortality occurred in larvae exposed during the first stadium, with only 3.8 percent surviving to adulthood (Figure 4b). Mortality also increased with increasing duration of exposure, with 72 percent and 68 percent surviving after 4 and 6 days of exposure respectively, compared to 81 percent survival for the control group (Table 6 and Figure 4a).

Development time for individuals exposed to nighttime treatment temperature 30°C increased with increasing duration but did not differ across stadia (Figure 5a, Table 7). Development time differed for individuals exposed to 32 or 34°C with respect to timing and duration of exposure (Figure 5b, c, Tables 8 and 9). Due to space limitations, each temperature was tested in separate rounds, each with its own control. Development times for controls across the three rounds were unequal, so we could not compare the effect of temperature on development time.

DISCUSSION

Exposure to extremely hot temperatures caused increased mortality in monarch larvae (Figure 1). Mortality increased slightly between 38°C and 40°C and substantially between 40°C and 42°C, regardless of the timing or duration of exposure. This suggests a physiological limit between 40 and 42°C. No larvae survived any exposure to 44°C.

Individuals in the third stadium showed increased mortality, particularly at 40°C when exposed for 6 days (Figure 1). If 42°C represents a physiological limit, this suggests that the third stadium itself poses additional susceptibility to heat stress. York and Oberhauser (2002) found mortality increases when third stadium individuals were exposed to 12 hour pulses of 36°C. Even if an individual survived the initial exposure at the third stadium, they were less able to pupate successfully (Figure 2). By this time, they were in control conditions, so this indicates a long-term effect of exposure to high temperatures.

Finally, individuals exposed at the fifth stadium for 6 days exhibit decreased survivorship at 40°C (Figure 1). This effect could result when individuals attempt to pupate while exposed to treatment conditions, or it could be the result of increased physiological stress from preparing to pupate coupled with heat stress. Individuals exposed to elevated temperatures during the fifth stadium for 4 or 6 days were also more likely to fall as they pupated (Figure 2).

The long-term impacts of heat stress continue, even if an individual survives to adulthood. Individuals exposed to increasing temperatures were lighter (Table 3). Small size may result in decreased male mating success (Solensky and Oberhauser 2009) and female fecundity (Oberhauser 1997). For the fall migrating generation, small size may

lead to decreased survivorship throughout the migration, winter and spring remigration (Masters et al. 1988, Arango 1996, Van Hook 1996, Alonso-Mejia et al. 1997).

Overall development time increased with exposure to increasingly elevated temperatures (Figure 3). As poikilotherms whose physiology is directly affected by external climatic conditions, monarchs should exhibit decreased development time with increasing temperatures. When individuals exposed to elevated temperatures take more degree days to develop to adulthood than control, this suggests that these elevated temperatures are negatively affecting their ability to develop.

When total degree days were recalculated to exclude daytime hours exposed to elevated temperatures, for most treatment combinations the total degree days did not differ from control, suggesting that their development stopped during exposure to elevated temperatures. But, the model in which we assumed that no development occurred during the heat treatment was less accurate for longer exposure times, suggesting that after a certain amount of time under unfavorable conditions, they cannot delay development or confine it to more favorable nighttime hours any longer (Figure 3c, 3d). Larvae exposed to 38°C in all stadia developed under hot conditions when exposed for 6 days, but when exposed to 40°C only those exposed during the fifth stadium developed in unfavorable conditions. The larvae may be able to gauge the risks of delaying development against the risks of developing in increasingly elevated temperatures, or they could be acclimating to the increased temperature.

When treatment temperatures were adjusted to calculate degree days, an adjusted temperature of 22°C removed any significant differences in development time between treatment and control individuals in the 38°C cohort. This indicates that when exposed to

daytime temperatures of 38°C, monarch larvae develop as slowly as if it were 22°C. For individuals exposed to 40°C during the day, no adjusted temperature completely removed all significant differences between treatment and control individuals. Still, 22°C eliminated most, leaving only first instars exposed for 6 days and third instars exposed for 4 days with longer development times than control. This further indicates that monarch larvae are particularly vulnerable to heat stress during these stadia.

By examining developmental gains throughout their life cycle, we determined that developmental delays largely occurred only while an individual was exposed to heat stress (Table 5). Once the immature monarch returned to control conditions, its development time returned to normal pace. We observed first stadium larvae under the shade of the container label and older larvae under leaves or under the filter papers at the bottom of the container. Individuals also seemed to eat less, if at all, under treatment conditions. These behaviors were recorded only anecdotally but suggest behavioral changes in response to elevated temperatures that should be explored further. These responses mimic immature monarch behavior under natural conditions, when larvae often hide under leaves or even in leaf litter during the hottest part of the day (Michelle Solensky, personal communication). Developmental delays resulting in additional time spent as an immature could lead to an increased risk of predation during a particularly vulnerable stage; the 24-hr mortality rate of first instar larvae is 40% (Alma DeAnda 2010) and less than 12% of eggs survive to the fifth instar (Oberhauser et al. 2001).

Increased nighttime temperatures also pose a risk to monarchs. York and Oberhauser (2002) showed that monarchs exposed to a constant 36°C died before adulthood, while single and repeated 12 hour pulses of 36°C show no increased mortality.

These findings, coupled our data showing survivorship up to 42°C when nighttime temperatures were 25°C, indicates that a decrease in temperature at night is necessary to cope with extreme temperatures. Nighttime temperatures up to 34°C did not lead to increased mortality in other treatments of short duration (1 or 2 days), suggesting that any decrease in temperature from daytime to nighttime is beneficial in the short term, as long as nighttime temperatures drop below the 36°C threshold reported by York and Oberhauser (2002) and Zalucki (1982). However, increased mortality occurred after 4 or 6 days of exposure to high nighttime temperatures (Figure 4).

Our findings indicate that, while monarchs can survive temperatures above the previously assessed limit of 36°C (York and Oberhauser 2002), mortality estimates should also consider other factors. Individuals exposed to elevated temperatures present a decreased ability to pupate without falling, decreased adult mass, and increased development time. There are serious effects of heat stress on monarch larvae making their ability to cope with climate change in question.²

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REFERENCES

- Alonso-Mejia, A., E. Rendon-Salinas, E. Montesinos-Patino and L. P. Brower. 1997. Use of lipid reserves by monarch butterflies overwintering in Mexico: implications for conservation. *Ecological Applications*. 7: 934-947.
- Arango, N. V. 1996. Stabilizing Selection in Migratory Butterflies: A Comparative Study of Queen and Monarch Butterflies. Masters thesis, Univ. of Florida (Gainesville).
- Batalden, R., K. Oberhauser and A. T. Peterson. 2007. Ecological niches in sequential generations of eastern North American monarch butterflies (Lepidoptera: Danaidae): the ecology of migration and likely climate change implications. *Env. Ent.* 36: 1365-1373.
- Crozier, L. G. 2004. Field transplants reveal summer constraints on a butterfly range expansion. *Oecologia*. 141: 148-157.
- DeAnda, A. 2010. The effect and importance of natural enemies on immature monarch (*Danaus plexippus*) populations. Masters thesis, Univ. of Minnesota (Twin Cities).
- Goehring, L. and K. S. Oberhauser. 2002. Effects of photoperiod, temperature, and host plant age on induction of reproductive diapause and development time in *Danaus plexippus*. *Ecol. Ent.* 27: 674-685.
- Goehring, L. and K. S. Oberhauser. 2004. Environmental factors influencing postdiapause reproductive development in monarch butterflies. in K. S. Oberhauser and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.
- Hartl FU. 1996. Molecular chaperones in cellular protein folding. *Nature* 381: 571–80.
- Holt, R. D. 1990. The microevolutionary consequences of climate change. *Trends Ecol. Evol* 5: 311-315.
- Huey RB. 1991. Physiological consequences of habitat selection. *Am. Nat.* 137: S91–115.
- IPCC. 2007. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Lindquist S. 1986. The heat-shock response. *Annu. Rev. Biochem.* 55: 1151–91

- Masters, A. R., S. B. Malcolm and L. P. Brower. 1988. Monarch butterfly (*Danaus plexippus*) thermoregulatory behavior and adaptations for overwintering in Mexico. *Ecology* 69: 458-467.
- Masters, A. R. 1993. Temperature and thermoregulation in the monarch butterfly. in S. B. Malcolm and M. P. Zalucki (eds.), *Biology and Conservation of the Monarch Butterfly*. Natural History Museum of Los Angeles County, Los Angeles, California.
- Monarch Larva Monitoring Project. 2011. www.mlmp.org.
- Oberhauser, K. S. 1997. Fecundity, Lifespan and Egg Mass in Butterflies: Effects of male-derived nutrients and females size. *Func. Ecol.* 11: 166-175.
- Oberhauser, K. S., M. D. Prysby, H. R. Mattila, D. E. Stanley-Horn, M. K. Sears, G. Dively, E. Olson, J. M. Pleasants, W. F. Lam and R. L. Hellmich. 2001. Temporal and spatial overlap between monarch larvae and corn pollen. *Proc. Natl. Acad. Sci.* 98: 11913-11918.
- Oberhauser, K. and A. T. Peterson. 2003. Modeling current and future potential wintering distributions of eastern North American monarch butterflies. *Proc. Natl. Acad. Sci.* 100: 14063-14068.
- Parmesan, C., N. Ryrholm, C. Stefanescu, J. K. Hill, C. D. Thomas, H. Descimon, B. Huntley, L. Kaila, J. Kullberg, T. Tammaru, et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*. 399: 579-583.
- Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37-42.
- Prysby, M. D. and K. Oberhauser. 2004. Temporal and Geographic Variation in Monarch Densities: citizen scientists document monarch population patterns, pp. 9-20. in K. S. Oberhauser and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.
- Solensky, M. J. and K. S. Oberhauser. 2009. Male monarch butterflies, *Danaus plexippus*, adjust ejaculates in response to intensity of sperm competition. *Anim. Beh.* 77: 465-472.
- Van Hook, T. 1996. Monarch Butterfly Mating Ecology at a Mexican Overwintering Site: Proximate causes of non-random mating. Ph.D. thesis, Univ. of Florida (Gainesville).

- York, H. A. and K. S. Oberhauser. 2002. Effects of duration and timing of heat stress on Monarch Butterfly (*Danaus plexippus*) (Lepidoptera: Nymphalidae) development. *J. Kans. Entomol. Soc.* 75: 290-298.
- Zalucki, M. P. 1981. The effects of age and weather on egg laying in *Danaus plexippus* L. (Lepidoptera: Danaidae). *Res. Popul. Ecol.* 23: 318-327.
- Zalucki, M. P. 1982. Temperature and rate of development in *Danaus plexippus* L. and *D. chrysippus* L. (Lepidoptera: Nymphalidae) *J. Aust. Entomol. Soc.* 21: 241-46.
- Zalucki, M. P. and R. L. Kitching. 1982. Temporal and spatial variation of mortality in field populations of *Danaus plexippus* L. and *D. chrysippus* L. larvae (Lepidoptera: Nymphalidae) *Oecologia* 53: 201-207.
- Zalucki, M. P. and W. A. Rochester. 2004. Spatial and temporal population dynamics of monarchs down-under: lessons for North America, pp. 219-228. In K. Oberhauser, and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.

Table 2.1: Model results for the analysis of survival probability by treatment temperature, timing and duration. Corresponds to Figure 2.1.

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	0.11	0.12	0.83	0.36
Temperature(40)	1.05	0.16	42.27	<0.0001
Temperature(42)	-3.02	0.22	183.06	<0.0001
Stadium(3)	-0.11	0.17	0.40	0.53
Stadium(5)	0.11	0.18	0.42	0.52
Duration(2)	0.43	0.23	3.53	0.06
Duration(4)	0.01	0.20	0.00	0.95
Duration(6)	-0.98	0.20	24.37	<0.0001
Temperature(40)*Stadium(3)	-0.54	0.22	6.02	0.01
Temperature(40)*Stadium(5)	0.40	0.24	2.74	0.10
Temperature(42)*Stadium(3)	0.12	0.31	0.14	0.71
Temperature(42)*Stadium(5)	-0.28	0.34	0.70	0.40
Stadium(3)*Duration(2)	-0.60	0.30	4.09	0.04
Stadium(3)*Duration(4)	0.61	0.28	4.56	0.03
Stadium(3)*Duration(6)	-0.75	0.29	6.86	0.01
Stadium(5)*Duration(2)	1.03	0.37	7.81	0.01
Stadium(5)*Duration(4)	-0.32	0.29	1.22	0.27
Stadium(5)*Duration(6)	-0.06	0.28	0.05	0.83

Table 2.2: Model results for the analysis of probability of falling by treatment timing and duration. Corresponds to Figure 2.2.

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	-0.71	0.12	36.79	<.0001
Temperature(40)	-0.12	0.11	1.01	0.31
Stadium(3)	0.14	0.16	0.69	0.41
Stadium(5)	0.40	0.16	6.16	0.01
Duration(2)	-0.15	0.19	0.58	0.45
Duration(4)	0.26	0.19	1.81	0.18
Duration(6)	0.27	0.22	1.50	0.22
Stadium(3)*Duration(2)	0.23	0.27	0.74	0.39
Stadium(3)*Duration(4)	0.15	0.26	0.31	0.58
Stadium(3)*Duration(6)	-0.85	0.32	6.89	0.01
Stadium(5)*Duration(2)	-0.68	0.27	6.16	0.01
Stadium(5)*Duration(4)	0.39	0.26	2.20	0.14
Stadium(5)*Duration(6)	0.99	0.30	10.96	0.001

Table 2.3: Male and female adult mass and right wing length by treatment temperature. Different letters indicate treatments that are significantly different from one another ($p < 0.05$).

Variable	Treatment	Male mass (grams) (SE)	Female mass (grams) (SE)	Male RWL (mm) (SE)	Female RWL (mm) (SE)
Temperature (°C)	38	0.59 (0.006)a	0.55 (0.007)a	52.40 (0.22)a	51.99 (0.23)a
	40	0.57 (0.007)b	0.52 (0.007)b	51.67 (0.24)b	51.23 (0.24)b
	42	0.52 (0.025)c	0.49 (0.023)b	49.98 (0.86)b	50.81 (0.83)ab
	44	-	-	-	-
Control	-	0.61 (0.02)a	0.57 (0.02)a	51.24 (0.64)ab	51.27 (0.55)ab

Table 2.4: Model results for the analysis of development time (growing degree days and adjusted growing degree days) by treatment temperature, timing and duration. Corresponds to Figure 2.3.

Development Time (Degree Days)					Adjusted Development Time (no heat)				Adjusted Development Time (22°C)			
Term	Estimate	Std Error	t Ratio	Prob>t	Estimate	Std Error	t Ratio	Prob>t	Estimate	Std Error	t Ratio	Prob>t
Intercept	339.43	0.70	481.90	0.00	295.50	0.70	424.76	0.00	305.75	0.70	439.49	0.00
Treatment(40)	5.15	0.70	7.32	<0.0001	3.59	0.70	5.15	<0.0001	3.59	0.70	5.15	<0.0001
Stadium (3)	0.66	1.06	0.62	0.53	0.71	1.04	0.68	0.50	0.71	1.04	0.68	0.50
Stadium (5)	-6.74	0.97	-6.96	<0.0001	-6.69	0.96	-6.99	<0.0001	-6.69	0.96	-6.99	<0.0001
Duration(2)	-11.53	1.17	-9.86	<0.0001	5.40	1.15	4.68	<0.0001	5.15	1.15	4.46	<0.0001
Duration(4)	6.08	1.16	5.23	<0.0001	-3.99	1.15	-3.47	0.00	5.76	1.15	5.01	<0.0001
Duration(6)	26.18	1.36	19.22	<0.0001	-10.88	1.35	-8.09	<0.0001	8.87	1.35	6.59	<0.0001
Treatment(40)*Stadium(3)	0.41	1.06	0.39	0.70	0.36	1.04	0.34	0.73	0.36	1.04	0.34	0.73
Treatment(40)*Stadium(5)	-0.39	0.97	-0.40	0.69	-0.45	0.96	-0.47	0.64	-0.45	0.96	-0.47	0.64
Treatment(40)*Duration(2)	0.64	1.17	0.54	0.59	1.21	1.15	1.04	0.30	1.21	1.15	1.04	0.30
Treatment(40)*Duration(4)	-0.82	1.16	-0.70	0.48	-1.25	1.15	-1.09	0.28	-1.25	1.15	-1.09	0.28
Treatment(40)*Duration(6)	5.74	1.36	4.22	<0.0001	4.31	1.35	3.21	0.00	4.31	1.35	3.21	0.00
Stadium(3)*Duration(2)	-2.24	1.73	-1.29	0.20	-2.29	1.71	-1.34	0.18	-2.29	1.71	-1.34	0.18
Stadium(3)*Duration(4)	6.73	1.66	4.05	<0.0001	6.68	1.64	4.07	<0.0001	6.68	1.64	4.07	<0.0001
Stadium(3)*Duration(6)	0.35	2.17	0.16	0.87	0.29	2.14	0.14	0.89	0.29	2.14	0.14	0.89
Stadium(5)*Duration(2)	3.79	1.60	2.37	0.02	3.74	1.58	2.36	0.02	3.74	1.58	2.36	0.02
Stadium(5)*Duration(4)	-4.54	1.64	-2.77	0.006	-4.60	1.62	-2.84	0.00	-4.60	1.62	-2.84	0.00
Stadium(5)*Duration(6)	-3.62	1.82	-1.99	0.047	-3.68	1.80	-2.05	0.04	-3.68	1.80	-2.05	0.04
Treatment(40)*Stadium(3)*Duration(2)	2.98	1.73	1.72	0.087	3.03	1.71	1.77	0.08	3.03	1.71	1.77	0.08
Treatment(40)*Stadium(3)*Duration(4)	0.62	1.66	0.37	0.71	0.68	1.64	0.41	0.68	0.68	1.64	0.41	0.68
Treatment(40)*Stadium(3)*Duration(6)	-3.05	2.17	-1.41	0.16	-3.00	2.14	-1.40	0.16	-3.00	2.14	-1.40	0.16
Treatment(40)*Stadium(5)*Duration(2)	-0.28	1.60	-0.17	0.86	-0.22	1.58	-0.14	0.89	-0.22	1.58	-0.14	0.89
Treatment(40)*Stadium(5)*Duration(4)	0.95	1.64	0.58	0.56	1.01	1.62	0.62	0.53	1.01	1.62	0.62	0.53
Treatment(40)*Stadium(5)*Duration(6)	-3.80	1.82	-2.09	0.038	-3.74	1.80	-2.08	0.04	-3.74	1.80	-2.08	0.04

Table 2.5: Development time (days) for each stadium by each factorial combination of treatment temperature, timing and duration. Shaded values represent time that larvae were in the heat treatment. Times are shown as mean days in each stadium. Treatment combinations separated by differing letters are significantly different ($p < 0.05$).

Tem P (°C)	Timing (Stadium)	Duration (Days)	Dev. time 1st Stadium (n)	Dev. time 2nd Stadium (n)	Dev. time 3rd Stadium (n)	Dev. time 4th Stadium (n)	Dev. time 5th Stadium (n)	Dev. time Pupa (n)	Total Dev. time (n)
C	C	C	2.1 (40) a	1.72 (39) a	1.87 (39) a	2.42 (38) abcde	3.74 (39) a	8.10 (36) ab	19.94 (36) a
38	1	1	2.79 (19) b	1.74 (19) a	1.94 (17) ab	2.29 (17) abcde	3.94 (19) abc	8.11 (18) ab	20.83 (18) bcdefg
		2	2.84 (19) b	1.94 (18) a	2.11 (17) ab	1.9 (17) ab	3.88 (17) ab	8.07 (15) abc	20.73 (15) abcdef
		4	3.05 (19) b	1.89 (19) a	2.00 (19) ab	2.32 (19) abcde	4.00 (19) abcd	8.06 (18) abc	21.33 (18) defg
		6	2.83 (18) b	1.83 (18) a	2.39 (18) ab	2.33 (18) abcdef	4.11 (18) abcde	8.00 (18) abcd	21.50 (18) defg
	3	1	-	-	1.94 (17) ab	2.41 (17) abcdef	4.13 (16) abcde	8.07 (15) abc	20.07 (15) abc
		2	-	-	1.79 (19) a	2.84 (19) cdef	4.05 (19) abcde	8.00 (18) abcd	19.94 (18) ab
		4	-	-	2.00 (19) ab	3.00 (19) cdef	4.63 (19) cdef	8.00 (18) abcd	21.39 (18) defg
		6	-	-	2.20 (19) ab	3.11 (19) def	4.80 (15) ef	7.79 (14) d	21.57 (14) defg
	5	1	-	-	-	-	4.17 (17) abcde	8.20 (15) a	20.00 (15) abc
		2	-	-	-	-	4.59 (17) bcdef	8.00 (17) abcd	20.12 (17) abc
		4	-	-	-	-	4.24 (17) abcde	8.21 (14) a	20.21 (14) abc
		6	-	-	-	-	4.29 (17) abcde	8.69 (16) e	20.94 (16) bcdefg
40	1	1	2.71 (17) b	1.71 (17) a	2.00 (16) ab	2.19 (16) abc	3.75 (16) a	8.07 (15) abc	20.47 (15) abcd
		2	2.83 (18) b	2.12 (17) a	1.95 (19) ab	2.26 (19) abcd	4.00 (19) abcd	8.00 (18) abcd	21.00 (18) cdefg
		4	2.80 (15) b	2.29 (14) ab	2.00 (14) ab	2.29 (14) abcdef	4.00 (14) abcde	8.00 (14) abcd	21.43 (14) defg

		6	2.84 (19) b	2.89 (18) b	2.69 (15) b	2.67 (15) abcdef	4.13 (15) abcde	8.00 (15) abcd	23.40 (15) h
		1	-	-	2.61 (17) b	1.82 (17) a	4.12 (17) abcde	8.20 (15) a	19.93 (15) ab
	3	2	-	-	2.53 (13) ab	2.77 (13) abcdef	4.00 (12) abcde	8.18 (11) a	21.00 (11) bcdefg
		4	-	-	2.61 (19) b	3.21 (19) f	4.22 (18) abcde	7.88 (17) bcd	21.82 (17) g
		6	-	-	2.59 (13) b	3.23 (13) ef	4.67 (6) abcdef	7.75 (4) bcd	22.25 (4) efgh
		1	-	-	-	-	4.56 (18) bcdef	8.18 (17) a	20.24 (17) abc
	5	2	-	-	-	-	5.05 (20) f	7.85 (20) cd	20.65 (20) abcde
		4	-	-	-	-	5.21 (19) f	8.00 (17) abcd	20.59 (17) abcde
		6	-	-	-	-	5.31 (13) f	8.67 (12) e	21.42 (12) defg
		1	3.80 (5) b	1.80 (5) ab	2.20 (5) ab	1.8 (5) abcdef	4.80 (5) abcdef	8.00 (4) abcd	22.50 (4) gh
	1	2	5.00 (1)	1.00 (1)	2.00 (1)	2.00 (1)	5.00 (1)	8.00 (1)	23.00 (1)
		4	-	-	-	-	-	-	-
		6	-	-	-	-	-	-	-
		1	-	-	2.60 (5) ab	3.20 (5) bcdef	4.20 (5) abcdef	7.8 (5) bcd	22.2 (5) fgh
42	3	2	-	-	3.00 (1)	5.00 (1)	3.00 (1)	8.00 (1)	23.00 (1)
		4	-	-	-	-	-	-	-
		6	-	-	-	-	-	-	-
		1	-	-	-	-	3.50 (2)	8.00 (2)	20.50 (2)
	5	2	-	-	-	-	5.33 (3) def	7.67 (3) cd	21.00 (3) abcdefg
		4	-	-	-	-	-	-	-
		6	-	-	-	-	-	-	-

Table 2.6: Model results for the analysis of survival by treatment duration, temperature and timing (temperatures indicate nighttime temperature; for all treatments day time temperature = 38°C). Corresponds to Figure 2.4.

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	1.13	0.13	79.52	<0.0001
Temperature(32)	0.47	0.16	9.06	0.0026
Temperature(34)	-1.07	0.19	30.06	<0.0001
Stadium(3)	0.49	0.16	9.13	<0.0025
Stadium(5)	0.82	0.17	23.53	<0.0001
Duration(2)	0.57	0.22	6.68	0.0097
Duration(4)	-0.57	0.18	10.33	0.0013
Duration(6)	-0.78	0.18	19.86	<0.0001
Temperature(32)*Stadium(3)	-0.88	0.21	17.34	<0.0001
Temperature(32)*Stadium(5)	-0.53	0.22	5.61	0.018
Temperature(34)*Stadium(3)	0.80	0.24	10.66	0.0011
Temperature(34)*Stadium(5)	1.41	0.27	28.24	<0.0001

Table 2.7: Model results for the analysis of development time (degree days) by treatment timing and duration. Corresponds to Figure 2.5a.

Term	Estimate	Std Error	t Ratio	Prob>t
Intercept	354.50	1.20	294.84	<0.0001
Stadium(3)	-2.68	1.67	-1.61	0.11
Stadium(5)	-1.25	1.70	-0.73	0.46
Duration(2)	-5.45	2.02	-2.70	0.0077
Duration(4)	4.43	2.14	2.07	0.039
Duration(6)	15.28	2.13	7.16	<0.0001

Table 2.8: Model results for the analysis of development time (degree days) by treatment timing and duration. Corresponds to Figure 2.5b.

Term	Estimate	Std Error	t Ratio	Prob>t
Intercept	348.84	1.13	309.68	<0.0001
Stadium(3)	0.19	1.65	0.12	0.91
Stadium(5)	-1.38	1.56	-0.89	0.38
Duration(2)	-10.17	1.84	-5.52	<0.0001
Duration(4)	6.01	1.95	3.08	0.0024
Duration(6)	21.15	2.14	9.88	<0.0001
Stadium(3)*Duration(2)	-2.45	2.62	-0.93	0.35
Stadium(3)*Duration(4)	8.72	2.80	3.11	0.0022
Stadium(3)*Duration(6)	-5.50	3.30	-1.67	0.0974
Stadium(5)*Duration(2)	7.29	2.57	2.84	0.0051
Stadium(5)*Duration(4)	-6.24	2.75	-2.27	0.025
Stadium(5)*Duration(6)	-2.67	2.83	-0.94	0.35

Table 2.9: Model results for the analysis of development time (degree days) by treatment timing and duration. Corresponds to Figure 2.5c.

Term	Estimate	Std Error	t Ratio	Prob>t
Intercept	360.23	1.98	181.93	<0.0001
Stadium(3)	7.19	1.98	3.63	0.0005
Duration(2)	-13.67	3.25	-4.21	<0.0001
Duration(4)	-0.080	3.57	-0.02	0.98
Duration(6)	38.02	3.64	10.45	<0.0001
Stadium(3)*Duration(2)	-0.92	3.25	-0.28	0.78
Stadium(3)*Duration(4)	-7.84	3.57	-2.20	0.03
Stadium(3)*Duration(6)	10.67	3.64	2.93	0.0042

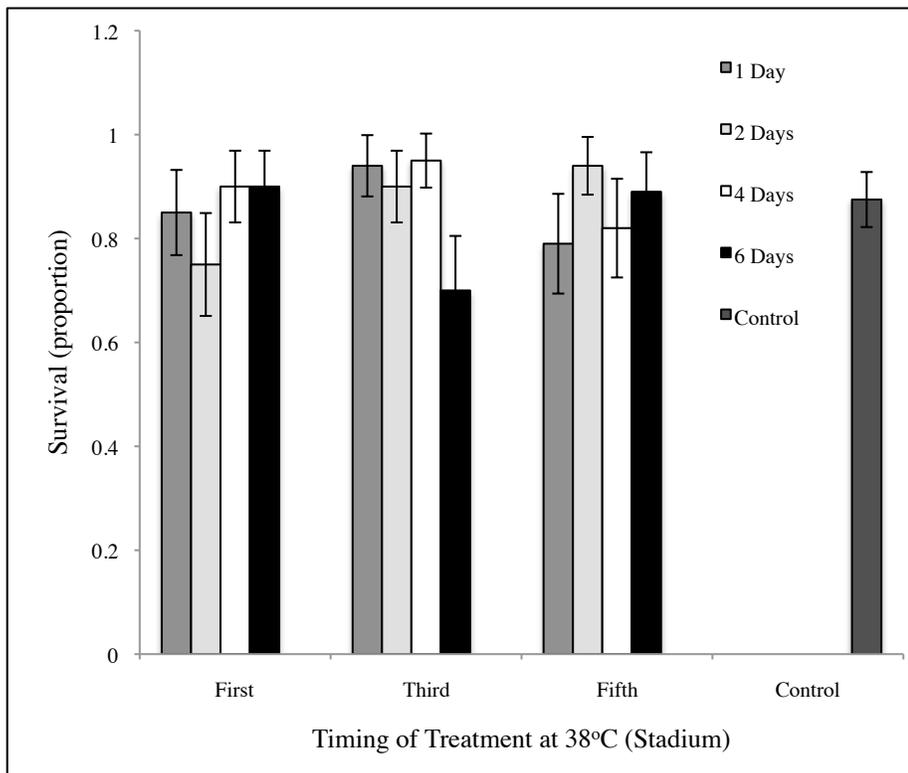


Figure 2.1a

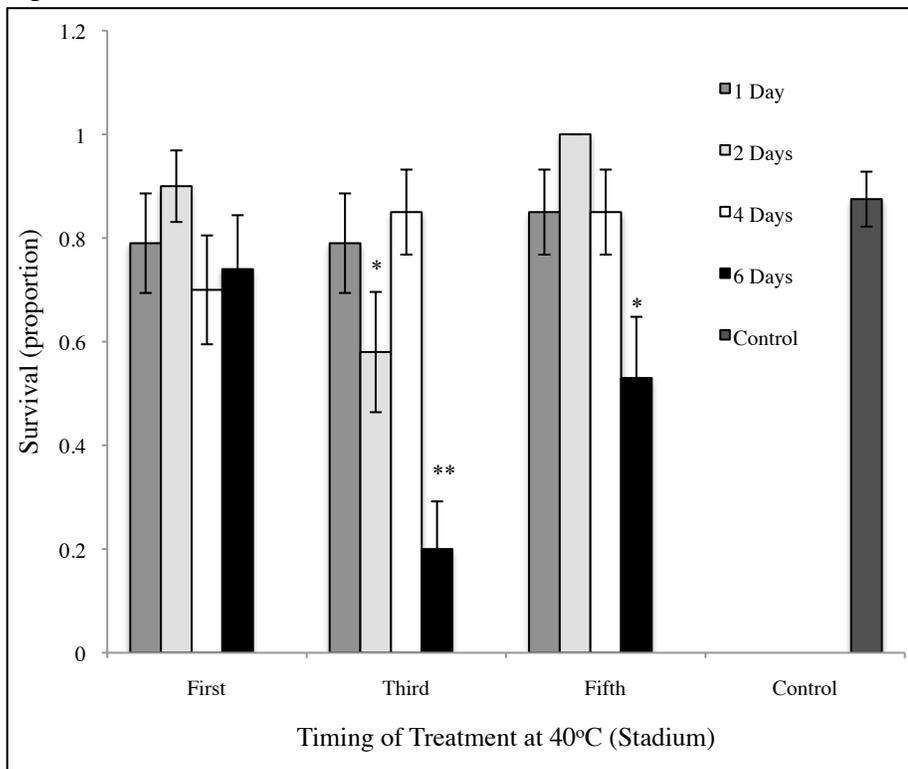


Figure 2.1b

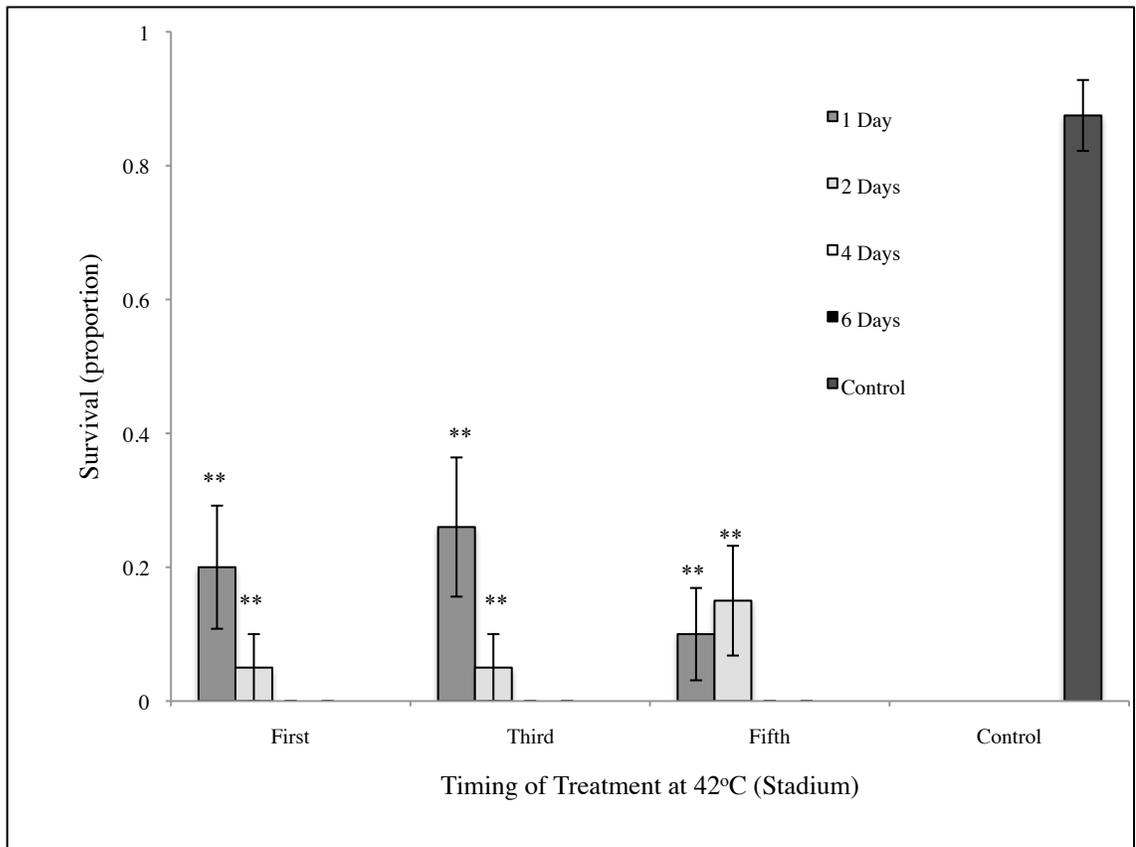


Figure 2.1c

Figure 2.1: Probability of survival by treatment timing and duration for 38°C (1a), 40°C (1b) and 42°C (1c) ($R^2=0.46$, d.f.=17, $\chi^2=444.76$, $p<0.0001$). * and ** indicates treatment combination is significantly different from control with $p<0.05$ and $p<0.01$, respectively.

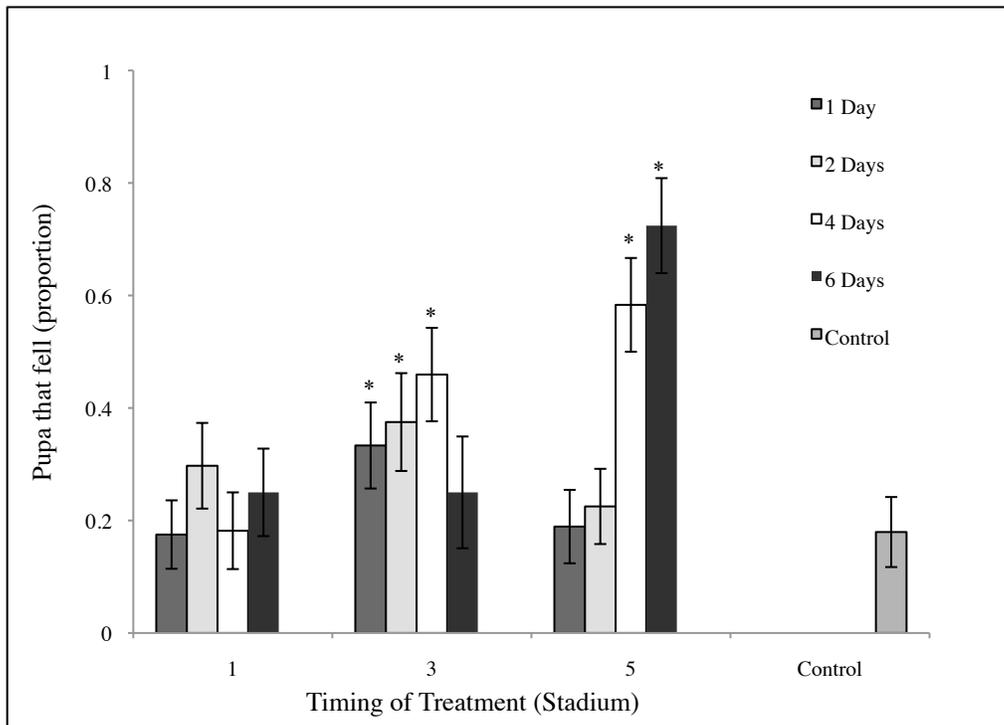


Figure 2.2: Probability pupa fell (and needed to be taped to container lid) by treatment timing and duration ($R^2 = 0.0895$, $\chi^2 = 45.50$, d.f. = 12, p-value < 0.0001). * indicates treatment combination is significantly different from control with $p < 0.05$.

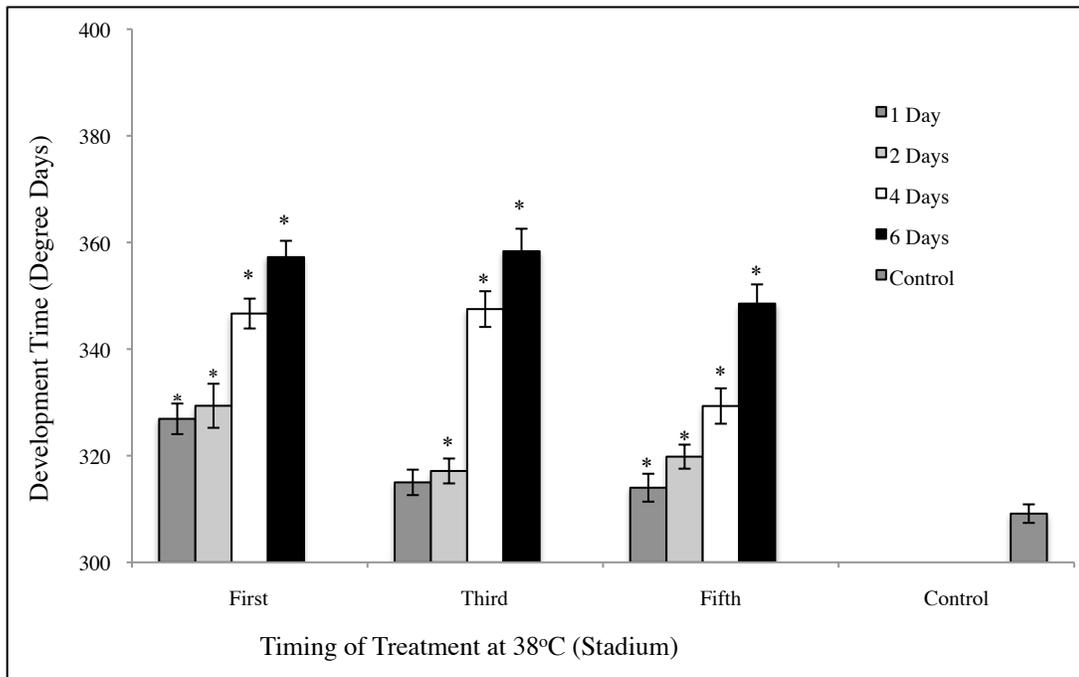


Figure 2.3a

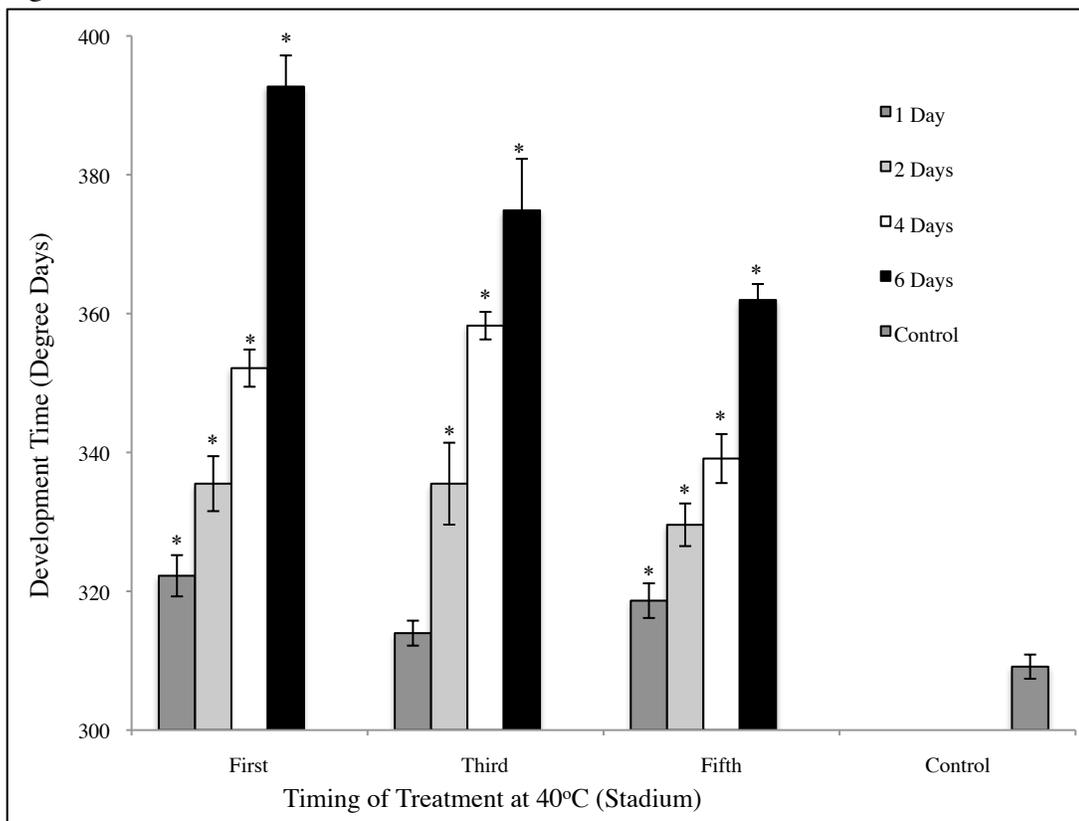


Figure 2.3b

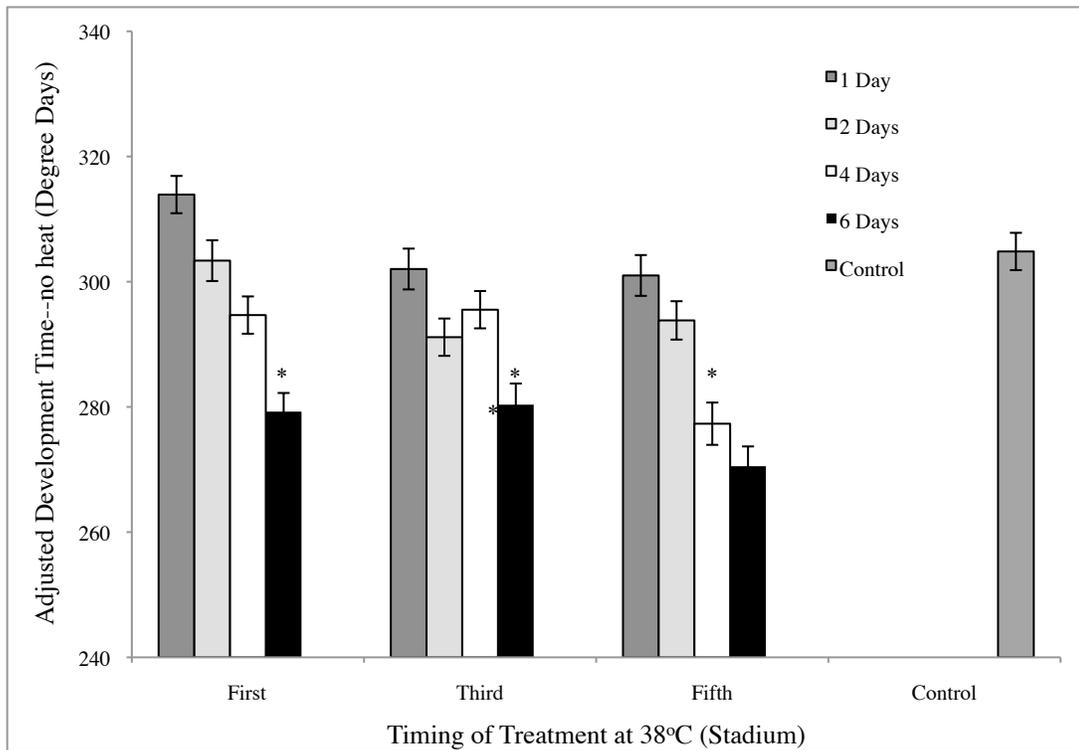


Figure 2.3c

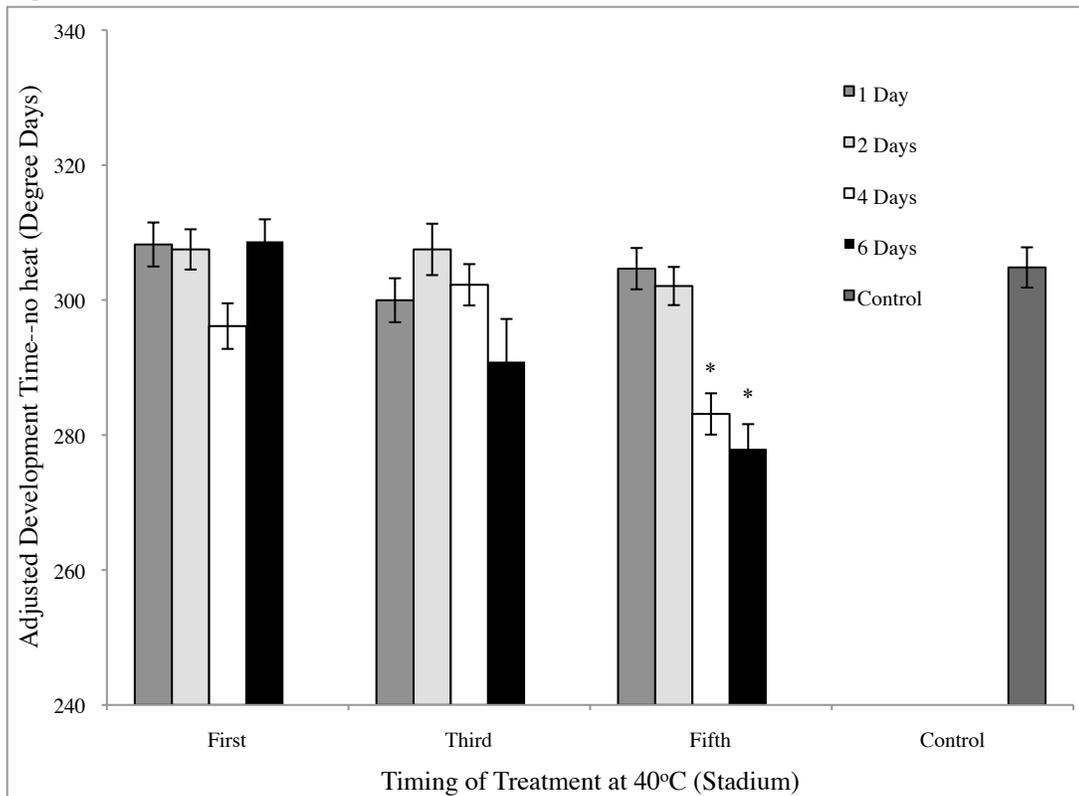


Figure 2.3d

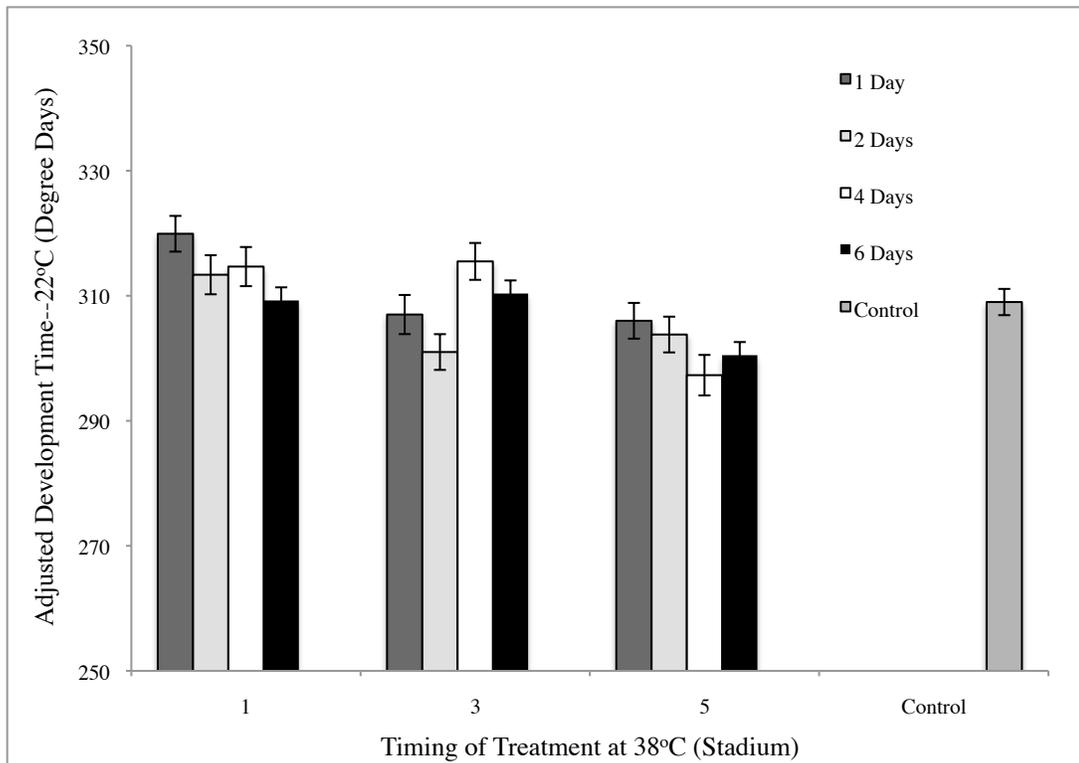


Figure 2.3e

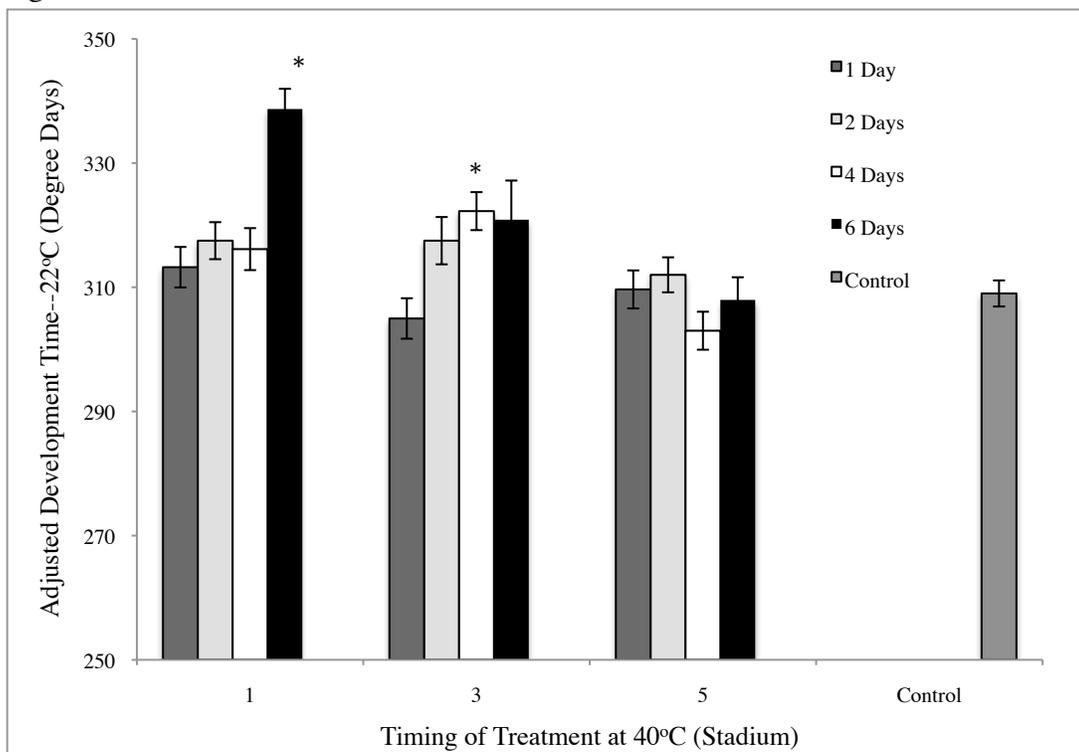


Figure 2.3f

Figure 2.3: Development time, measured in degree days, by treatment timing and duration at 38°C (a) and 40°C (b) ($F = 36.19$, d.f. = 23, 347, p-value = <0.0001). Adjusted development time, measured by not including time spent at elevated temperatures, by treatment timing and duration at 38°C (c) and 40°C (d) ($F = 13.42$, d.f. = 23, 347, p-value = <0.0001). Adjusted development time, calculated by substituting 22°C for both elevated temperatures of 38°C and 40°C, by treatment timing and duration at 38°C (e) and 40°C (f) ($F = 19.62$, d.f. = 23, 347, p-value = <0.0001). * indicates treatment combination is significantly different from control with $p < 0.05$.

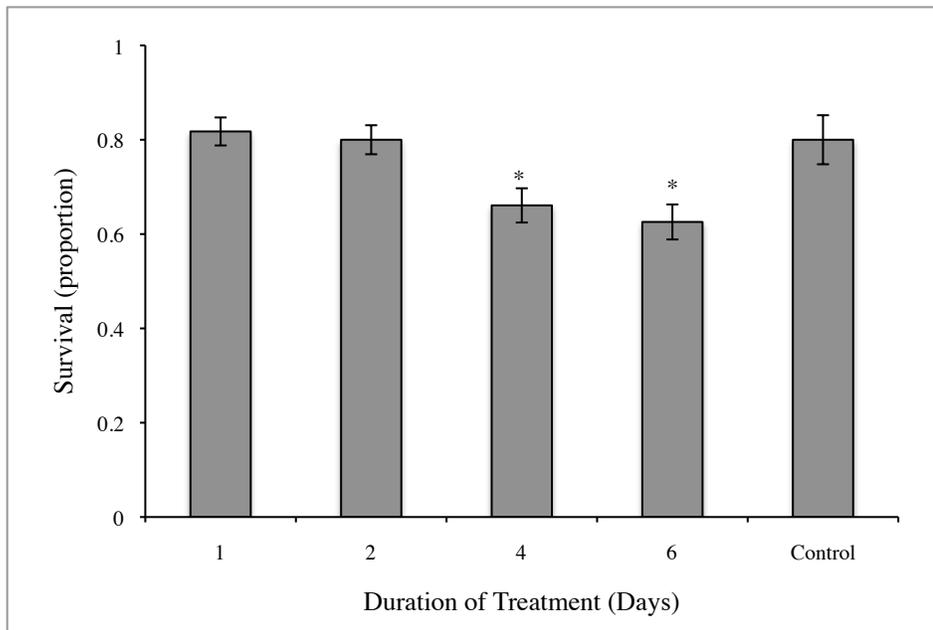


Figure 2.4a

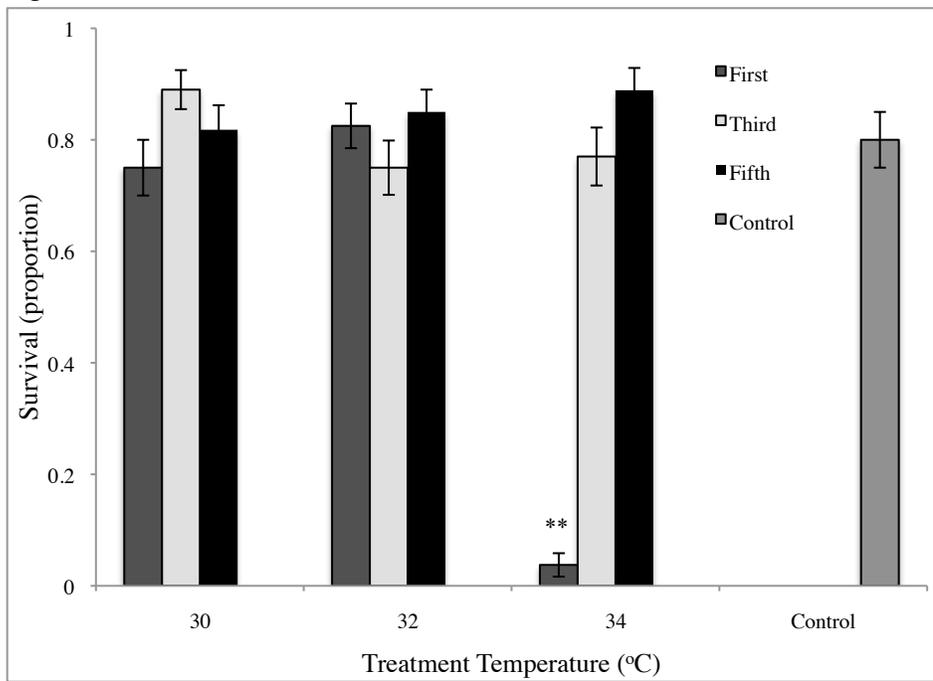


Figure 2.4b

Figure 2.4: Probability of survival by duration (a) and by temperature and timing (b) ($R^2=0.315$, d.f.=11, $\chi^2=252.63$, $p<0.0001$). * and ** indicates treatment combination is significantly different from control with $p<0.05$ and $p<0.01$, respectively.

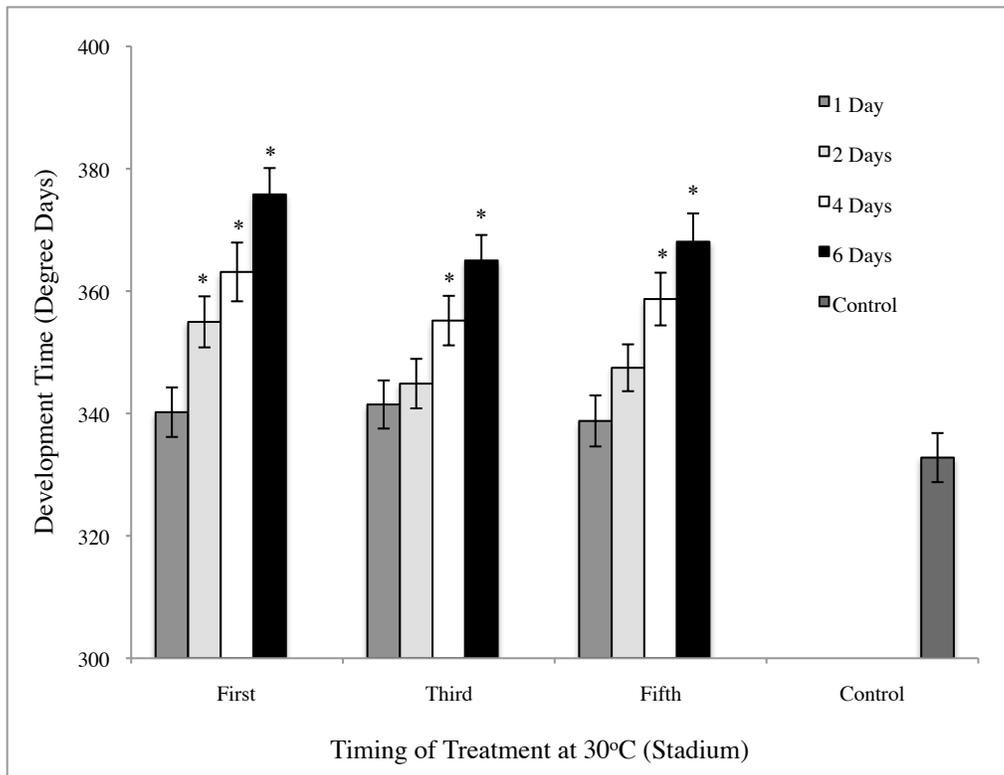


Figure 2.5a

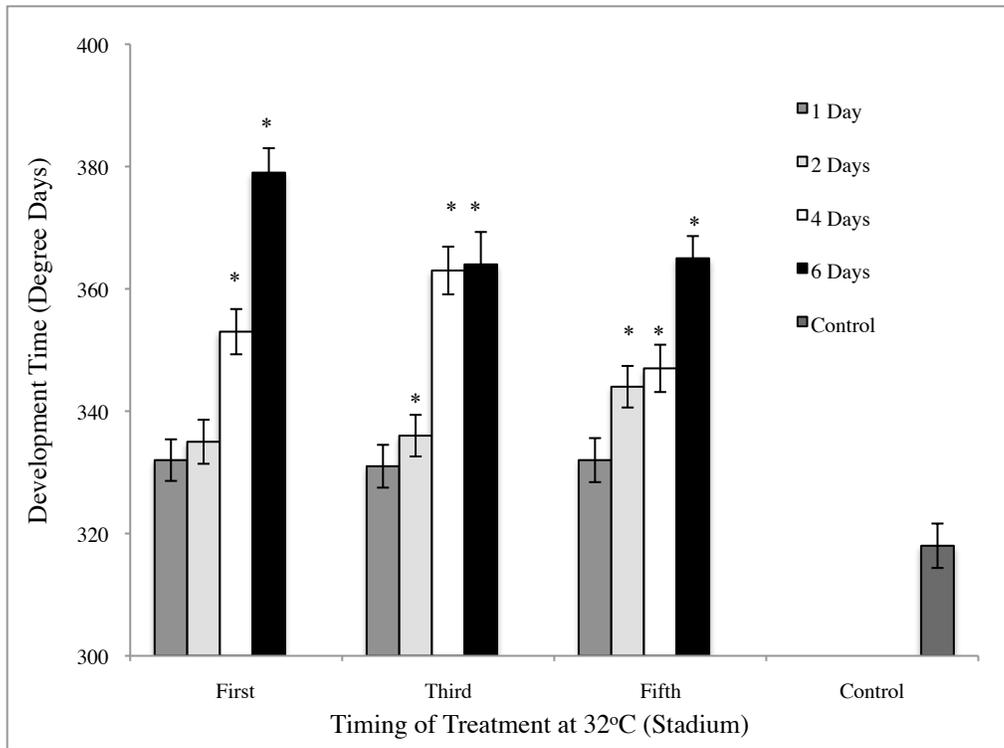


Figure 2.5b

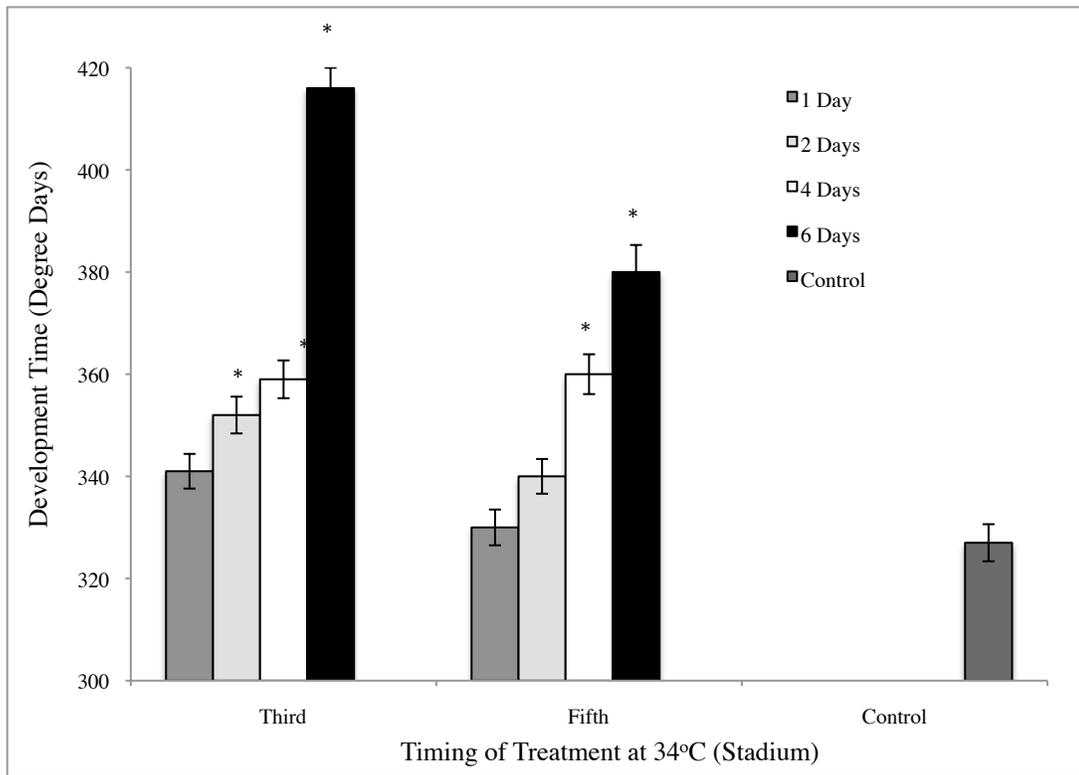


Figure 2.5c

Figure 2.5: Development time (degree days) by treatment timing and duration at nighttime temperatures of 30°C (a) ($F=17.83$, d.f.=5, 185, $p<0.0001$), 32°C (b) ($F=17.61$, d.f.=11.182, $p<0.0001$) and 34°C (c) ($F=20.79$, d.f.=7, 93, $p<0.0001$). Daytime temperatures for all treatments were 38°C.

CHAPTER 3

Potential Changes to the Eastern North American Monarch Butterfly Migration in Response to an Introduced Milkweed, *Asclepias curassavica*

SUMMARY

Climate change threatens the monarch butterfly migration; hotter summers may necessitate a longer and faster migration and the overwintering grounds in Mexico may become unsuitable. To assess the monarch butterfly's potential to cope with a changing climate and adjust its migration, I aimed to identify any level of plasticity currently present in the migration. The Monarch Larva Monitoring Project (MLMP), a citizen-science program, indicates eggs and larvae present in Texas in the fall when monarchs are migrating supposedly in a state of reproductive arrest, diapause. Here, I investigate whether some monarchs are breaking diapause in Texas in response to an introduced milkweed, *Asclepias curassavica*, or whether some are failing to enter diapause in their natal grounds and are migrating while reproducing. I analyzed MLMP data throughout the monarch migration flyway, surveyed native and introduced milkweeds in Texas and exposed migratory butterflies to native and introduced milkweeds to assess their reproductive response. MLMP data show a correlation between latitude and a second peak in eggs, suggesting that monarchs could be laying eggs as they travel south, supporting the hypothesis that some butterflies are not in diapause while migrating. MLMP data and my field studies also suggest that the introduced milkweed is in better

health and preferred by egg-laying females in the fall, indicating that it could be influencing migrating females. The reproductive response experiment suggested that only a small proportion of the population may be responding to the changing milkweed cues, but as the entire population funnels through Texas on its way to the overwintering habitat, any perturbations here could have population wide effects. This research indicates that there is variability in monarch migratory and reproductive behavior that could allow this species to adjust to future climate change.

INTRODUCTION

Each fall, monarch butterflies undertake one of the most well-known insect migrations. As they fly up to 4500 km from their breeding grounds throughout eastern North America to their overwintering habitat in Mexico, most butterflies are thought to be in a state of reproductive diapause (Brower 1985, 1995). They will remain in this juvenile state throughout the winter, until they travel north in the spring to lay eggs in the southern United States. Being in diapause allows individuals to conserve as much energy as possible and to survive the long migration and winter (Herman 1981, Herman and Tatar 2001), and allows them to put off reproduction until conditions are favorable.

The monarch migration is threatened by climate change. Current over-wintering sites in Mexico are predicted to be unsuitable for monarchs in 50 years (Oberhauser and Peterson 2003). Already, a January 2002 winter storm marked by extreme cold and precipitation caused the most severe winter kill of monarchs known, with 70-80% mortality in two major over-wintering colonies (Brower et al. 2004), and the frequency of

such storms is predicted to increase with climate change (Oberhauser and Peterson 2003). Ecological niche models predict severe northward shifts in the location of summer breeding habitat, especially during July and August, when the population is probably increasing in size prior to the fall migration (Batalden et al. 2007). This will necessitate a longer and faster migration, and with the availability of overwintering sites in a changed climate in question, the Eastern North American monarch population is likely to need some level of plasticity in their migration to ensure their survival.

There is some evidence that monarchs are capable of changing their migration patterns and distributions over relatively short time-scales (Vane-Wright 1991). Monarchs extended their range in the mid-19th century to islands in the South Pacific and arrived on Australia's east coast around 1871 (Vane-Wright 1991, Zalucki and Clarke 2004, Clarke and Zalucki 2004). Until recently, many monarchs in Australia migrated to avoid hot and dry summers (Dingle et al. 2000). Now increased irrigation from suburban sprinklers allows milkweed to persist throughout the summers, and some monarchs in Perth and Adelaide no longer migrate (Zalucki and Rochester 1999).

A similar phenomenon could be occurring in Texas. Monarchs are thought to be absent throughout the summer and winter, yet Urquhart (1987) documented fall reproduction in the southern United States and recent citizen-science monitoring efforts show adults, eggs and larvae present in the fall and winter after being absent through most of the summer (Prysby and Oberhauser 2004, Howard et al. 2010). Two non-exclusive hypotheses could explain this fall breeding behavior. First, some monarchs may fail to enter diapause in their natal grounds and lay eggs as they migrate southward, as

they do as they move northward during the spring migration. Calvert (1999) suggests this scenario to explain the presence of eggs in the fall. Migratory behavior and diapause are not always coupled (Perez and Taylor 2004, Zhu et al. 2009), allowing for the first hypothesis. Second, some monarchs could be breaking diapause in response to environmental cues as they migrate. Many gardens and parks in Texas contain *Asclepias curassavica*, a species of milkweed native to Mexico, but not Texas. Because these gardens are often watered and fertilized throughout the hot Texas summer, this introduced milkweed persists into the fall, remaining healthy and possibly attractive to migrating monarchs. This newly-available fall milkweed could provide environmental cues that prompt some individuals to reproduce. Here, I investigate these two hypotheses and attempt to determine whether this fall Texas generation continues the migration to Mexico or remains to overwinter in Texas. Whether these fall reproducing monarchs represent a shift due to an already changing climate or due to changes in biotic cues, this behavior could indicate a level of plasticity in the monarch migration that will allow them to cope with further environmental perturbations.

There is a third scenario where some monarchs are staying in Texas throughout the summer, continuing to breed into the fall. However, since I am most interested in changes to the fall migration, I will focus on individuals who are migrating and the two hypotheses outlined above.

To test the first hypothesis that some monarchs are laying eggs as they migrate, I used data from the Monarch Larva Monitoring Program (MLMP) to examine seasonal patterns of egg densities and milkweed abundance across states along their migratory

route. To test the second hypothesis that introduced milkweed is affecting monarch fall breeding, I also used MLMP data, coupled with a field study in Texas, to compare egg densities, milkweed health and immature survival at MLMP sites with and without *Asclepias curassavica*. Finally, I conducted experiments to assess adult and immature monarch responses to milkweed quality and availability in Texas during the fall migration, and to determine if monarchs stay in Texas through the winter or continue the migration to Mexico.

METHODS

MLMP Data

MLMP methods are described elsewhere (Prysby and Oberhauser 2004, www.mlmp.org). Those most relevant to this study are the methods for assessing egg densities; volunteers survey a random selection of milkweed each week at a site of their choosing (or survey all of their milkweed on smaller sites) and record the numbers of eggs or larvae by stadium observed on these plants. Monitoring sites include gardens with planted milkweed, natural areas, restored prairies, and other areas such as roadside ditches. While they do not record the species of milkweed on which each monarch is observed, they do report the species of milkweeds available at their site. They also report management practices (fertilizing, weeding, and planting).

I used MLMP data to identify peak weeks of egg density (weeks that are preceded and followed by multiple weeks of lower values) in three regions in the monarchs' central flyway (Howard and Davis 2009): northern, middle, and southern. I calculated weekly

regional egg densities by dividing the total number of eggs observed by MLMP volunteers in a given region in a given week by the number of milkweed plants surveyed. In most cases, these values are summed across years. I excluded weeks within regions if fewer than three monitoring events occurred over all years. I identified peak weeks of egg density across the following states, chosen because they represent the monarch's central flyway and have many MLMP monitoring sites: MN, WI, IA (northern region), IL, MO, NE (middle region), and TX (southern region). Typically, when monarchs first arrive in an area, egg density is very high, probably due to limited milkweed availability. This marks the beginning of the first generation, and a second peak signals the second generation (or second and third generation combined in the Upper Midwestern US). This second peak in the Upper Midwestern US has been used as a general indicator of monarch population size (Karen Oberhauser, personal communication). With the exception of sites in TX, I only used MLMP site/year combinations when there were continuous data from the monarchs' spring arrival until the second peak, ensuring that I was analyzing a true peak and not an artifact of erratic monitoring. However, in TX many volunteers fail to monitor in the hot summer months, so this criterion would exclude most sites. MLMP data from a typical TX monitoring location show a high first peak in the spring, tapering off into nothing throughout the summer, then another peak in the fall. I only included sites in TX that monitored at least one week before and after the fall peak, again to ensure that I was analyzing a true peak in monarch abundance.

I also used MLMP data to estimate the change in relative availability of native milkweed species and the introduced *A. curassavica* over the course of a season in Texas.

I identified 11 Texas sites that were monitored at minimum from April through November in a single year. I calculated the mean number of plants examined per monitoring session at each site for the entire year and the mean number examined per session in each month, and divided the monthly mean by the yearly mean to obtain relativized values. I then compared the relative abundance of milkweed on sites that did and did not have *A. curassavica* to determine if these sites had relatively more milkweed at different times over the course of the year.

Field Study

The methods I used followed closely the methods for MLMP volunteers. I surveyed 14 sites in fall 2007, all within 230 km of San Antonio, Texas. I assessed monarch egg and larval densities on every milkweed plant at each site. I recorded a condition rating for each plant with categorical values of 1 = <5, 2 = 5-40, 3 = 41-80, and 4 = 81-100 percent yellowed or dying leaves. I estimated herbivory damage for each monitored plant with another categorical scale of 1 = 0, 2 = <5, 3 = 5-25, and 4 = >25 percent eaten leaves. I also measured plant height and the immediate milkweed density for each plant (the number of milkweeds within a square meter).

I collected all monarch eggs and larvae observed on native and non-native milkweed, and reared them outdoors in a shady spot in a residential yard near San Antonio, Texas. Larvae were grouped by collection site and placed in either a deli container (500 ml) with a ventilated lid, a large plastic bin (6 l) with a mesh top or a mesh cage (0.67 x 0.67 x 0.67 m), depending on the age and number of individuals per group. Larvae were fed leaves from collected and purchased *A. curassavica* plants. When they

emerged as adults, the monarchs were shipped overnight to Minnesota. Immediately upon arrival, I placed them in mesh cages in a greenhouse with 16-hours of light per day to assess their reproductive status. If males mated, they were considered reproductive. Males that did not mate were considered to be in diapause. If females mated and subsequently laid eggs, they were considered reproductive. Those that did not lay eggs were to be dissected to determine if their ovaries contained developed oocytes. They had *ad libitum* access to sponges soaked with 20% honey-water while they were in the mating cages.

Assessing diapause maintenance in fall monarchs

I raised approximately 500 monarchs from eggs laid by wild-caught individuals captured in St. Paul, MN in summer 2007. They were reared outdoors in mid-September—early-October in wooden and mesh cages (0.3 x 0.5 x 0.7 m) with about 50 individuals per cage. Every day, larvae received fresh cuttings of common milkweed, and their cages were cleaned. Their outdoor location exposed them to the decreasing daylight and fluctuating temperatures known to induce diapause (Goehring and Oberhauser 2002). They emerged as adults between October 8 and October 15th and were either carried by plane or shipped overnight to Texas. On October 15th, I collected 253 roosting adults from a stand of trees located approximately 250 km west of San Antonio, Texas. Monarchs were given unique numbers on their hind wings with sharpie markers so that I could distinguish individuals.

The MN-reared and wild-caught butterflies were randomly placed in one of four 2 x 2 x 2 m mesh cages (approximately 150 individuals per cage, with equal numbers of

males and females). One cage contained no milkweed, a second contained wild-growing native milkweed *A. asperula*, a third contained wild-growing *A. oenotheroides*, and the last contained a non-native milkweed *A. curassavica* purchased from a local organic nursery. All cages contained established native Texas grasses and were clustered within approximately 20 square feet of one another. Butterflies had *ad libitum* access to sponges filled with 20% honey-water and remained in the cages for 11 days. I measured their reproductive status as described above.

All analyses were conducted in JMP v. 5.1.2.

RESULTS

Phenological patterns

Monarchs' arrival in the spring is marked by a large influx of eggs in each region (Figure 1). While MLMP volunteers only measure milkweed density once per season, the number of plants they monitor provides an index of the number of plants available in their site, and early milkweed abundance is low relative to later abundance (Figure 2). Thus, the high per plant densities in the spring probably reflect low milkweed abundance rather than high monarch abundance (Figure 1). Monarchs arrive in the south in March and lay eggs, with a spring peak in egg density in mid-March. The low values in the south from April through August show that monarchs are largely gone during these months, (except in sites with the introduced *A. curassavica*, see below). The adults that result from the southern eggs fly northward, laying eggs in the mid-region and then the northern region. Monarchs are largely gone from the middle region through June and

July. In the northern part of the flyway, a second peak in egg density follows closely on the first, indicating a second generation (or combined second and third generation, Figure 1). At lower latitudes (the middle and southern part of the flyway) another peak often follows the period during the hottest part of the summer where few or no eggs are observed (Figure 1).

The timing of the second generation of eggs across MN, WI, IA, MO, NE, IL and TX is correlated with latitude (Figure 3). Around 45 degrees latitude (MN and WI), the second peak of eggs occurs, on average, at week 29, the last week in July. At 38 degrees latitude (MO), the second peak occurs in week 32, the second week in August. At some MLMP sites, these eggs are laid after a period of absence, suggesting that they are offspring from monarchs arriving from the north. Finally, in TX (30 degrees latitude), the second peak of eggs occurs at during week 39, the second week in September. Again, these eggs appear after a period of absence, and some volunteers report eggs through December, suggesting that multiple fall generations are produced at some sites (Figure 1). The higher proportion of eggs per plant after week 45 suggests limited milkweed supply (Figure 2), much like in the spring.

Phenological patterns of milkweed

A. curassavica is more likely to be in sites that were planted, regularly fertilized and weeded (Table 1). Interviews with MLMP volunteers suggest that native milkweed senesces during the summer and only returns in the fall during years with sufficient rainfall, while *A. curassavica* in maintained areas is green throughout the growing season. The relative abundance of milkweed in sites with and without *A. curassavica*

across the season shows strikingly different patterns. Milkweed abundance in sites with only native species peaks in the spring but remains low through the fall, while milkweed abundance increases in abundance through May and then remains relatively stable throughout the fall in sites with *A. curassavica* (Figure 4, see month by A.c interactions in Table 2). The ratio of monthly mean milkweed numbers to yearly mean milkweed is also predicted by the previous month's precipitation (Table 2). In particular, fall 2008 and 2009 were extremely wet in Texas; the effects of these wet years are illustrated in Figure 4, where data for sites without *A. curassavica* in 2008-2009 and 2001-2007 are separated. In August 2008, San Antonio, the nearest large city to sites analyzed for milkweed quantity, received 4.98 inches of rain. The following month, there was a significant spike in native milkweed counts, blurring the distinction between relativized native and non-native milkweed abundance. In October 2009, San Antonio received 11.9 inches of rain and the following November saw a similar increase in native milkweed.

Effects of milkweed species on monarch phenology and survival

The type of milkweed in a site affected monarch use of the site. MLMP volunteers reported more eggs per plant at Texas sites with *A. curassavica* than at sites without this introduced milkweed, however, this effect depends on month (Table 3). In March, when monarchs typically are returning from overwintering in Mexico, there is no preference for MLMP sites with *A. curassavica* (Figure 5). Beginning in April, when monarchs typically leave the southern United States, MLMP data show significantly more eggs per milkweed plant at sites with *A. curassavica* for the remainder of the year ($p < 0.05$). The number of eggs per plant at sites without tropical milkweed declines from

0.66 in March to 0.13 in April and remains significantly lower than the March value throughout the year ($p < 0.05$). The number of eggs per plant at sites with tropical milkweed is never significantly different from the March value ($p > 0.05$) (Figure 5).

Across all of the sites that I surveyed in the fall of 2007, I found an average of 1.09 eggs per *A. curassavica* plant and an average of 0.016 eggs per native milkweed plant (Figure 6). *A. curassavica* was in significantly better condition than the native milkweeds, with average condition ratings of 1.24 and 2.09 respectively (Table 4). There was no difference in the amount of herbivory damage, indicating that neither milkweed was preferred earlier in the season or by other insects. *A. curassavica* plants surveyed were considerably taller than the native species and thus contained more leaf area. This difference could explain the additional eggs found on each plant, but the significant difference in egg counts remained when weighted by plant height ($p < 0.0001$). *A. curassavica* also occurs in higher density (Table 4). With the introduced milkweed being denser and in better condition, *A. curassavica* presence could simply attract more monarchs to these MLMP sites. However, two of the sites I surveyed contained both introduced and native milkweeds; in these sites there were more eggs on *A. curassavica* than native milkweed, indicating that females are choosing not just the sites but the plants themselves (Figure 6). Additionally, females did not lay more eggs on native milkweed in sites where *A. curassavica* also occurred than at sites with only native milkweed ($R^2 = 0.0038$, $F_{1,127} = 0.49$, $p = 0.49$).

Reproductive condition of fall migrants

The probability of mating did not differ between the MN-raised monarchs and monarchs collected roosting in Texas with 2 out of 119 wild and 9 out of 167 MN male monarchs mated, respectively (Pearson's chi-square=2.58, d.f.=1, 284, p=0.11). Also, the number of days until a monarch mated did not depend on butterfly origin with wild-caught butterflies mating an average of 4.5 days (range 4-5 days), and MN butterflies an average of 5.25 days (range 3-8 days) after being put into the cage (t-test, $t=-0.59$, d.f.= 9, p=0.57). Given these data, I assumed no difference in reproductive state at the start of this experiment, with both cohorts being in diapause and migratory. In addition, during the experiment most butterflies congregated in the southwest corner of the mating cages. They did not track the sun throughout the day, as non-migratory summer butterflies do (personal observations), but stayed in this corner. I did not quantify movement, but observed that these butterflies rarely flew around the cage, or landed on the milkweed or other plants, as I have observed in summer cage experiments.

No monarchs mated in the cage without milkweed or in the cage with *A. asperula*, but 6 of 77 and 5 of 90 of the males mated in the cages with *A. curassavica*, and *A. oenotheroides* respectively. Examining all four cages separately, there were significant differences in the probability of mating between the four cages (Pearson chi-square=17.06, d.f.=3, 581, p=0.0007), but no significant differences between the probability of mating in the cage with *A. curassavica* and *A. oenotheroides* (Pearson chi-square=0.213, d.f.=1, 336, p=0.64) (Figure 7).

None of the 57 wild-caught monarchs reared in outdoor conditions in Texas and mailed to Minnesota mated in a greenhouse cage with extended daylight conditions.

DISCUSSION

There is clearly reproduction by monarchs moving into Texas in the fall (Calvert 1999, Prysby and Oberhauser 2004), but this analysis, using data from the MLMP, provides the first quantification of the degree to which this occurs, and its correlation with the presence of non-native milkweed. This is a departure from the traditional model of the monarchs' fall migration, in which monarchs are thought to be in reproductive diapause through the migration and winter only to mate and lay eggs in the spring (Brower 1995).

Two hypotheses could explain breeding during the fall migration. First, a subset of the eastern North American migratory monarch population may migrate south in a reproductive, non-diapause state. The timing of monarch arrival in the Mexican overwintering sites, and the fact that the vast majority of monarchs that arrive Mexico are in reproductive diapause (Herman 1981, Herman and Tatar 2001) demonstrate that most migratory monarchs are in diapause. However, migratory behavior is not necessarily linked to diapause (Perez and Taylor 2004, Zhu et al. 2009), so some butterflies could lay eggs along the migratory route as they do during the spring migration. Figure 3 shows a wave of eggs (and thus reproductive monarchs) moving south from mid-July through mid-September, supporting this hypothesis. The individuals laying these eggs could

represent a level of plasticity that could allow this population of monarchs to cope with additional pressures of climate change.

Data from the mating cages suggest that monarchs are capable of breaking diapause during the fall migration, and provide some insights into the cues that trigger diapause development and the degree to which this occurs. It is very likely that the MN-reared and wild-caught butterflies in the mating cages were in diapause and migratory at the start of the experiment, given the 4-5 days until they started mating and their clustering behavior in the southwest corner of the cages. The observed mating is likely to be in response to milkweed presence, as there was no mating observed in the cage without milkweed (Figure 8). Goehring and Oberhauser (2004) showed that the presence of milkweed can hasten diapause development at the end of the overwintering period, and these findings suggest that this may be an effective cue in the fall as well.

MLMP data and my survey data indicate a preference for *A. curassavica* during the fall, and it is possible that the presence of this species is more likely to trigger diapause development. However, *A. oenotheroides* had a similar effect, complicating this interpretation (Figure 8). There are inherent limitations of my experimental design. The conditions in the mating cages deviate from those a monarch would normally encounter; the butterflies were held in close proximity to host plant for an extended period of time. Typically, a monarch in diapause would be likely to contact host plants during brief encounters. Additionally, MLMP data suggest that fewer native milkweed plants are available in the fall than in the spring (Figure 4) and the field data show that *A. curassavica* plants are in better condition than native species (Table 4), making it less

probable that a migratory monarch encounters a native host plant in good condition.

Thus, simply by being available and healthy enough to be included in this experiment, the native plants in these cages were by definition in better condition than typical native plants in the fall. More work is needed to further quantify the role *A. curassavica* might play in diapause development; at minimum, these data suggest that the presence of healthy milkweed is correlated with mating behavior.

The fact that such a low proportion of individuals mated in the mating cage experiment (Figure 7) suggests that a small proportion of the population responds to the presence of this cue, but because all butterflies on their way to the Mexican overwintering grounds move through this region, any environmental perturbations here could have population wide effects.

MLMP data and my field data show further differences between *A. curassavica* and native species. *A. curassavica* had more eggs per milkweed plant (Figures 5 and 6). Monarchs could be choosing to lay eggs on this introduced milkweed because it is in better condition than native species (Table 4). Also, MLMP sites with *A. curassavica* are more likely to be planted, fertilized and weeded (Table 1). In my surveys, I encountered several instances of MLMP volunteers protecting their *A. curassavica* plants and the monarchs feeding on them. One volunteer kept her plants in pots so she could bring them inside if it got too cold. Another had tied several fallen pupae onto the sturdy stems of *A. curassavica* plants. With this care, the *A. curassavica* plants grow much taller than the native plants (Table 4) and more closely resemble a large shrub. Thus, it appears that

monarchs choose to lay more eggs on this introduced milkweed species, it remains in better condition through the fall, and it could be affecting monarchs' fall migration.

The offspring of these fall breeding individuals could 1) enter a state of reproductive diapause and join the wintering population in Mexico, 2) remain in the southern U. S. in reproductive diapause, or 3) breed in the southern U. S. It is likely that all three of these outcomes are occurring. The fact that none of the individuals reared outdoors in Texas in the fall mated in a Minnesota greenhouse suggests that they were at the very least in diapause, and while this does not guarantee their migratory status, it seems likely at least some of them would continue the journey to Mexico. Indeed, observers in Mexico report that there is often a second influx of individuals in December (Eduardo Rendon, personal communication). Additional work should be done to analyze the effect that these individuals' late start has on their survival until spring, but Monarch Watch data suggests that the later a monarch is tagged in the fall, the less likely it is to be recovered in Mexico (O. Taylor, personal communication).

We do know that monarchs can remain in the southern U. S. throughout the winter; observers in Virginia Beach, VA tagged a wild monarch on 25 September 2005 and recovered the same individual on 2 March 2006 (Journey North 2011). It is highly unlikely that a breeding individual could live long, so this individual was probably in diapause. Additionally, Howard et al. (2010) documented overlap of immature and adult monarchs along the Gulf Coast and up to the Georgia-South Carolina border, but only adult monarchs on the South Carolina, North Carolina, and south Virginia coasts. This suggests (but does not prove) that at least some of these individuals were in diapause.

The presence of immature monarchs in Texas and along the Gulf Coast every month throughout the winter (Howard et al. 2010 and Figure 1) demonstrates that at least some of the offspring of fall breeding individuals remain in this region and reproduce. This strategy, and thus the presence of *A. curassavica*, could present a significant threat to individuals in some years. *A. curassavica* cannot tolerate freezing temperatures; the plant may remain viable underground and grow again under favorable conditions, but any foliage exposed to a freeze will senesce. Thus, even if a monarch larva survived the freeze itself, it would not have milkweed to eat. The average low temperature in January in Dallas is 32.7 degrees Fahrenheit; San Antonio, 39.8 degrees Fahrenheit; and Houston, 42.9 degrees Fahrenheit (Office of the Texas State Climatologist). With these average lows, a freeze could occur in a significant portion of winters, making monarch survival unlikely.

Further work is needed to determine the degree to which monarchs migrating southward in the fall, and the offspring of those southward migrants that reproduce, respond to environmental cues. This research suggests that there is variability in monarch migratory and reproductive behavior that may be affected by climate and milkweed quality and presence. This variability is likely to have individual fitness consequences, and could also indicate a level of plasticity within the monarch migratory population that could allow this species to adjust to future environmental perturbations, including climate change.³

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REFERENCES

- Batalden, R., K. Oberhauser and A. T. Peterson. 2007. Ecological niches in sequential generations of eastern North American monarch butterflies (Lepidoptera: Danaidae): the ecology of migration and likely climate change implications. *Env. Ent.* 36: 1365-1373.
- Brower, L. P. 1985. New perspectives on the migration biology of the monarch butterfly, *Danaus plexippus* L. in M. A. Rankin (ed.), *Migration: Mechanisms and Adaptive Significance*. Contrib. Univ. Texas Marine Science.
- Brower, L. P. 1995. Understanding and misunderstanding the migration of the monarch butterfly (Nymphalidae) in North America: 1857-1995. *J. Lepid. Soc.* 49:304-85.
- Brower, L. P., D. R. Kust, E. Rendon-Salinas, E. G. Serrano, K. R. Kust, J. Miller, C. Fernandez del Ray, and K. Pape. Catastrophic Winter Storm Mortality of Monarch Butterflies in Mexico in January 2002. in Oberhauser, K. S. and M. J. Solensky. 2004. *Monarch Butterfly Biology and Conservation*. Cornell University Press.
- Calvert, W. H. 1999. Patterns in the spatial and temporal use of Texas milkweeds (Asclepiadaceae) by the monarch butterfly (*Danaus plexippus* L.) during fall, 1996. *J. Lep. Soc.* 53: 37-44.
- Clarke, A. R. and M. P. Zalucki. 2004. Monarchs in Australia: on the winds of a storm? *Bio. Inv.* 6: 123-127.
- Dingle, H., W.A. Rochester and M.P. Zalucki. 2000. Relationships among climate, latitude and migration: Australian butterflies are not temperate-zone birds. *Oecologia* 124:196-207.
- Goehring, L. and K. S. Oberhauser. 2002. Effects of photoperiod, temperature, and host plant age on induction of reproductive diapause and development time in *Danaus plexippus*. *Ecol. Ent.* 27: 674-685.

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- Goehring, L. and K. S. Oberhauser. 2004. Environmental factors influencing postdiapause reproductive development in monarch butterflies. in K. S. Oberhauser and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.
- Herman, W. S. 1981. Studies on the adult reproductive diapause of the monarch butterfly, *Danaus plexippus*. *Biol. Bull.* 160:89-106.
- Herman, W. S. and M. Tatar. 2001. Juvenile hormone regulation of longevity in the migratory monarch butterfly. *Proc. R. Soc. Lond. B* 268: 2509-2514.
- Howard, E. and A. K. Davis. 2009. The fall migration flyways of monarch butterflies in eastern North America revealed by citizen scientists. *J. Insect Conserv.* 13: 279-286.
- Howard, E., H. Aschen, A. K. Davis. 2010. Citizen science observations of monarch butterfly overwintering in the southern United States. *Psyche*. Vol. 10, Article ID 689301, 6 pages.
- Journey North: A Global Study of Wildlife Migration. 2011. <http://www.learner.org/jnorth/>.
- Monarch Larva Monitoring Project. 2011. www.mlmp.org.
- Oberhauser, K. and A. T. Peterson. 2003. Modeling current and future potential wintering distributions of eastern North American monarch butterflies. *Proc. Natl. Acad. Sci.* 100: 14063-14068.
- Office of Texas State Climatologist, Texas A&M University. <http://atmo.tamu.edu/osc/>.
- Perez, S. M. and O. R. Taylor. 2004. Monarch butterflies' migratory behavior persists despite changes in environmental conditions, pp. 85-88. in K. S. Oberhauser and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.
- Prysby, M. D. and K. Oberhauser. 2004. Temporal and Geographic Variation in Monarch Densities: citizen scientists document monarch population patterns, pp. 9-20. in K. S. Oberhauser and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.
- Urquart, F. A. 1987. *The Monarch Butterfly: International Traveler*. Nelson-Hall, Chicago, IL.

- Vane-Wright, R. I. 1991. The Columbus hypothesis: an explanation for the dramatic 19th century range expansion of the monarch butterfly. in S. B Malcolm and M. P. Zalucki (eds.), *Biology and Conservation of the Monarch Butterfly*. Natural History Museum of Los Angeles County, CA.
- Zalucki, M. P. and A. R. Clarke. 2004. Monarchs across the Pacific: the Columbus hypothesis revisited. *Bio. Jour. Linn. Soc.* 82: 111-121.
- Zalucki, M. P. and W. A. Rochester. 1999. Estimating the effect of climate on the distribution and abundance of the monarch butterfly, *Danaus plexippus* (L.): a tale of two continents, pp. 151-163. In J. Hoth, L. Merino, K. Oberhauser, I. Pisanty, S. Price, and T. Wilkinson (eds.), *The 1997 North American Conference on the Monarch Butterfly*. Commission for Environmental Cooperation, Montreal, Canada.
- Zhu, H., R. J. Gegear, A. Casselman, S. Kanginakudru and S. M. Reppert. 2009. Defining behavioral and molecular differences between summer and migratory monarch butterflies. *BMC Bio* 7: 14.

Table 3.1: Management practices at Texas MLMP sites with and without *A. curassavica*.

	Percent Planted	Percent Fertilized	Percent Weeded	Sample Size
Sites with <i>A.</i> <i>curassavica</i>	0.88	0.38	0.67	24
Sites without <i>A.</i> <i>curassavica</i>	0.15	0.04	0.19	26

Table 3.2: Model results for the analysis of normalized milkweed counts for Texas sites with and without *A. curassavica* from 2001-2009. Corresponds to Figure 3.4.

Term	Estimate	Std Error	t Ratio	P-value
Intercept	0.79	0.06	12.80	<.0001
Prev. Month				
Precip.	0.05	0.01	4.13	<.0001
No A. c.	-0.07	0.05	-1.33	0.19
Month 3	-0.47	0.25	-1.86	0.06
Month 4	0.14	0.16	0.87	0.39
Month 5	0.66	0.12	5.39	<.0001
Month 6	0.45	0.12	3.68	<.0001
Month 7	0.12	0.12	1.02	0.31
Month 8	-0.24	0.14	-1.77	0.08
Month 9	0.00	0.12	-0.02	0.98
Month 10	-0.07	0.14	-0.50	0.61
Month 11	-0.27	0.14	-1.93	0.06
No A. c. *				
Month3	0.13	0.25	0.53	0.60
No A. c. *				
Month4	0.38	0.16	2.39	0.02
No A. c. *				
Month5	0.48	0.12	3.95	<.0001
No A. c. *				
Month6	0.34	0.12	2.78	0.01
No A. c. *				
Month7	-0.10	0.12	-0.85	0.40
No A. c. *				
Month8	-0.33	0.14	-2.40	0.02
No A. c. *				
Month9	-0.44	0.12	-3.59	<.0001
No A. c. *				
Month10	-0.10	0.14	-0.70	0.48
No A. c. *				
Month11	-0.15	0.14	-1.09	0.28

Table 3.3: Model results for the analysis of the number of eggs per milkweed plant at MLMP sites in Texas. Corresponds to figure 3.5.

Term	Estimate	Std Error	t Ratio	p-value
Intercept	0.28	0.025	11.30	<0.0001
No A. c.	-0.16	0.025	-6.60	<0.0001
Month 4	0.0050	0.056	0.09	0.93
Month 5	0.0084	0.054	0.16	0.88
Month 6	-0.11	0.060	-1.84	0.067
Month 7	-0.13	0.066	-2.04	0.042
Month 8	-0.13	0.067	-1.94	0.052
Month 9	-0.03	0.062	-0.54	0.59
Month 10	-0.04	0.064	-0.55	0.58
Month 11	0.10	0.08	1.25	0.21
Month 12	-0.009	0.13	-0.97	0.94
No A. c. *				
Month 4	0.0059	0.056	0.11	0.92
No A. c. *				
Month 5	-0.11	0.054	-2.08	0.04
No A. c. *				
Month 6	0.022	0.060	0.37	0.71
No A. c. *				
Month 7	0.038	0.066	0.57	0.57
No A. c. *				
Month 8	0.080	0.067	1.19	0.24
No A. c. *				
Month 9	0.069	0.062	1.11	0.27
No A. c. *				
Month 10	-0.00031	0.064	0.00	0.99
No A. c. *				
Month 11	-0.21	0.082	-2.55	0.011
No A. c. *				
Month 12	-0.097	0.13	-0.77	0.44

Table 3.4: Summary of milkweed quality from field study. Average condition rating of *A. curassavica* is significantly better than native milkweed ($F_{1,172}=24.14$, $p<0.0001$).

Average herbivory rating does not differ between *A. curassavica* and native milkweed ($F_{1,172}=0.15$, $p=0.70$). The average height for *A. curassavica* is taller than the average height for native milkweed ($F_{1,172}=339.27$, $p<0.0001$). *A. curassavica* has a higher average density than native milkweed ($F_{1,172}=9.47$, $p=0.0024$).

	Average Condition Rating (se)	Average Herbivory Rating (se)	Height (cm) (se)	Density (#milkweeds/m ²) (se)	Sample Size
Native Milkweed	2.09 (0.09) a	2.34 (0.10) a	27.22 (1.59) a	2.55 (0.27) a	129
<i>A.</i> <i>curassavica</i>	1.24 (0.15) b	2.27 (0.17) a	84.69 (2.69) b	4.16 (0.45) b	45

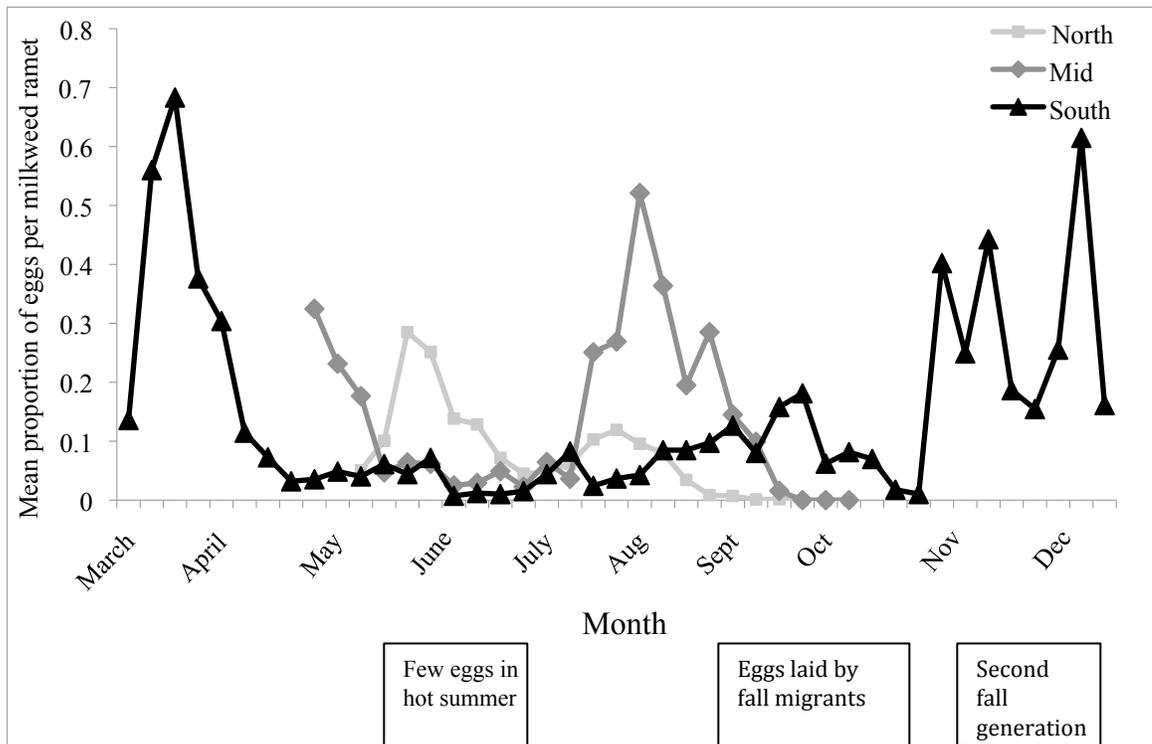


Figure 3.1: Mean eggs density by week at MLMP sites in Minnesota, Wisconsin and Iowa (North, 215 sites), Missouri, Illinois and Nebraska (Mid, 17 sites) and Texas (South, 52 sites) for all years combined, 1997-2009. Tick marks are weeks 10-52, but labeled as month for easier reference. Text boxes below refer only to the egg densities in TX. Weeks 10-16 show the arrival of overwintering monarchs to lay their eggs in the south, a few monarchs persist throughout the hot, dry summers, and others return in the fall around week 38. The high egg densities in the south late in the year are likely due to low milkweed abundance.

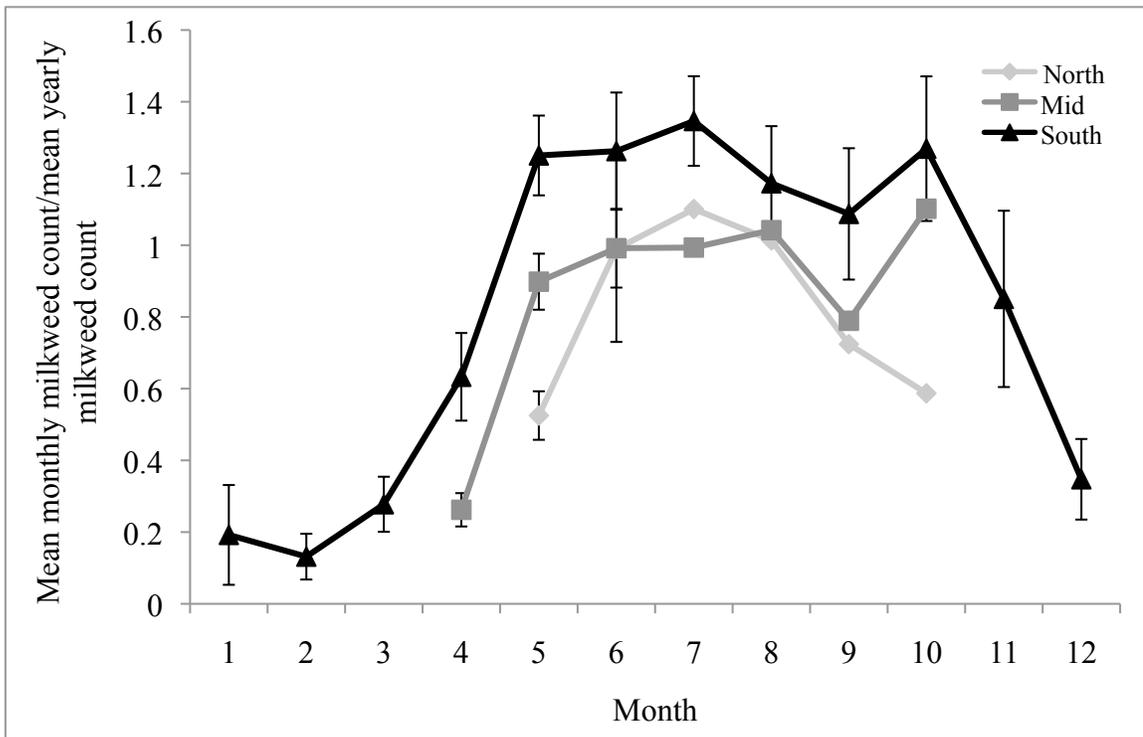


Figure 3.2: Normalized milkweed abundance by month at MLMP sites in Minnesota, Wisconsin and Iowa (North), Missouri, Illinois and Nebraska (Mid) and Texas (South).

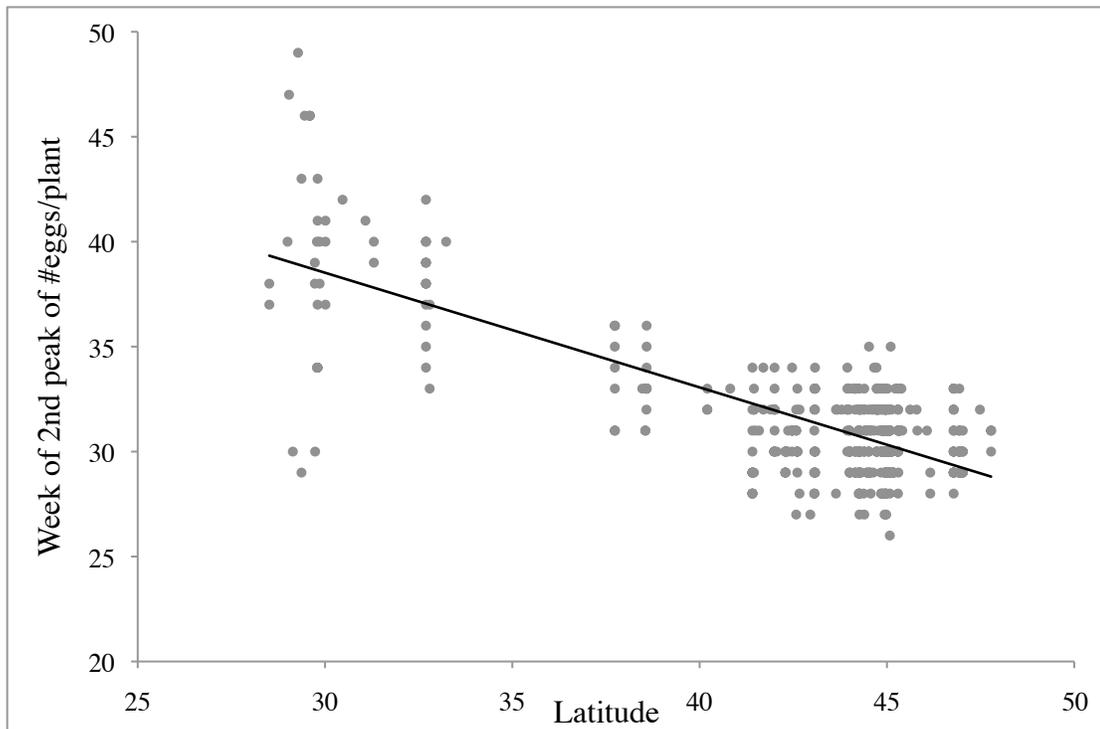


Figure 3.3: Week of peak egg abundance after the first spring peak. MLMP data from MN, IA, WI, IL, MO, NE, and TX. Each data point represents the peak week for a single MLMP site in a single year. Linear regression equation: $\text{week} = 53.19 - 0.53 \cdot \text{latitude}$ ($R^2 = 0.568$, $F_{1,420}$ ratio = 552.64, $p < 0.0001$).

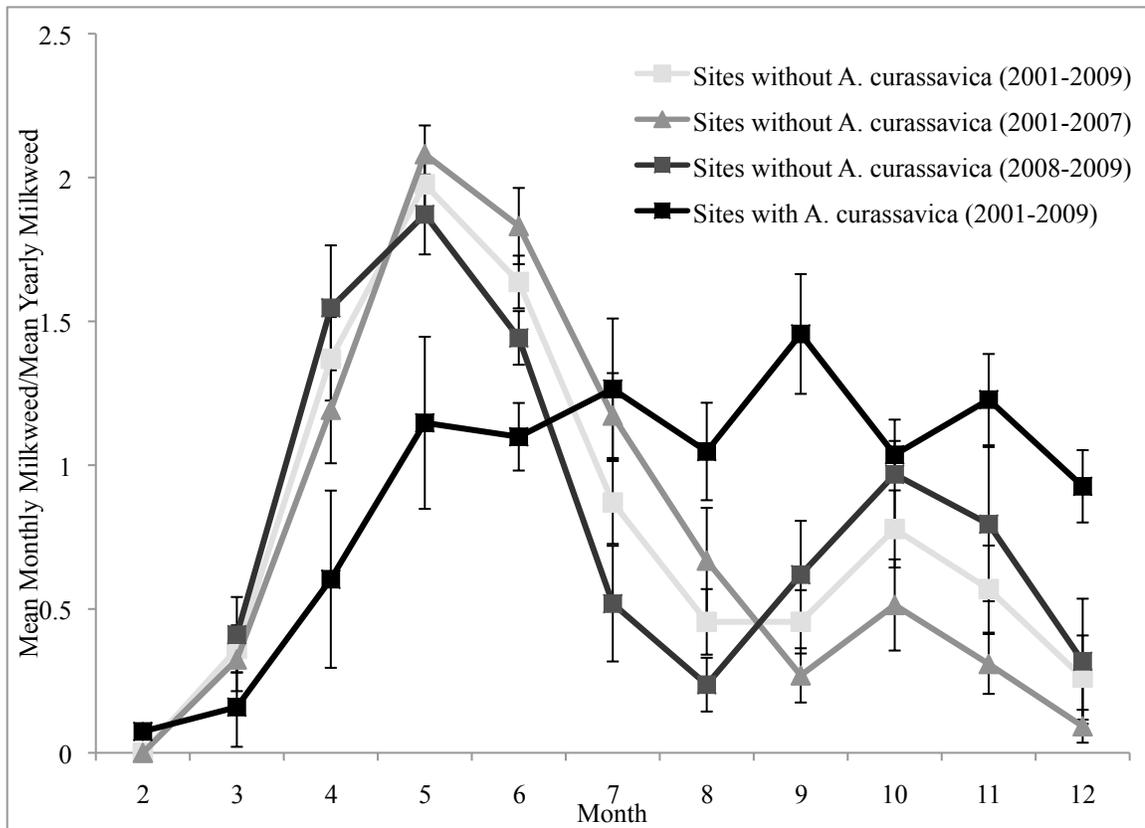


Figure 3.4: Normalized milkweed abundance in Texas. Ratio of mean monthly milkweed counts to mean yearly milkweed counts in sites, across months and in the presence and absence of *A. curassavica* ($R^2 = 0.633$, $F_{20, 159} = 11.96$, $p < 0.001$). Also shown are sites without *A. curassavica* from 2001-2007 and from 2008-2009, as precipitation was markedly higher in years 2008 and 2009. Error bars are standard error.

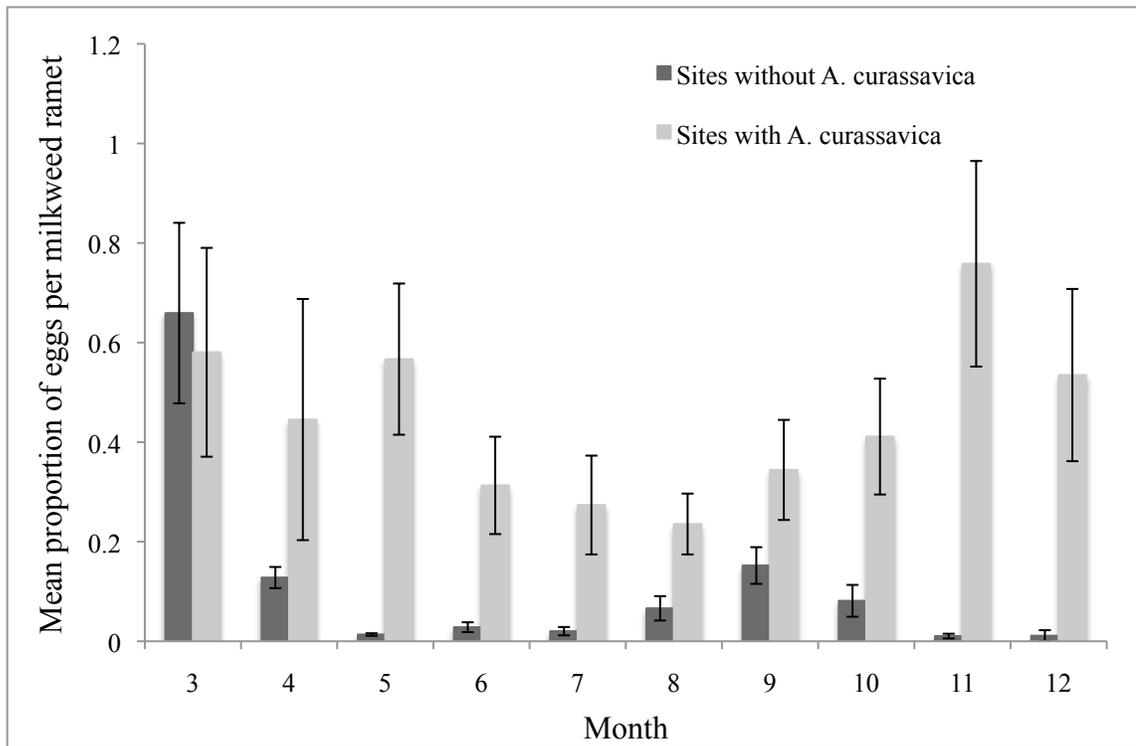


Figure 3.5: Mean proportion of eggs per milkweed plant in Texas MLMP sites with and without *A. curassavica* throughout the year. Month and *A. curassavica* presence correlated with egg density ($R^2 = 0.095$, $F_{19, 1155}$ ratio = 6.38, $p < 0.0001$). Error bars represent standard error.

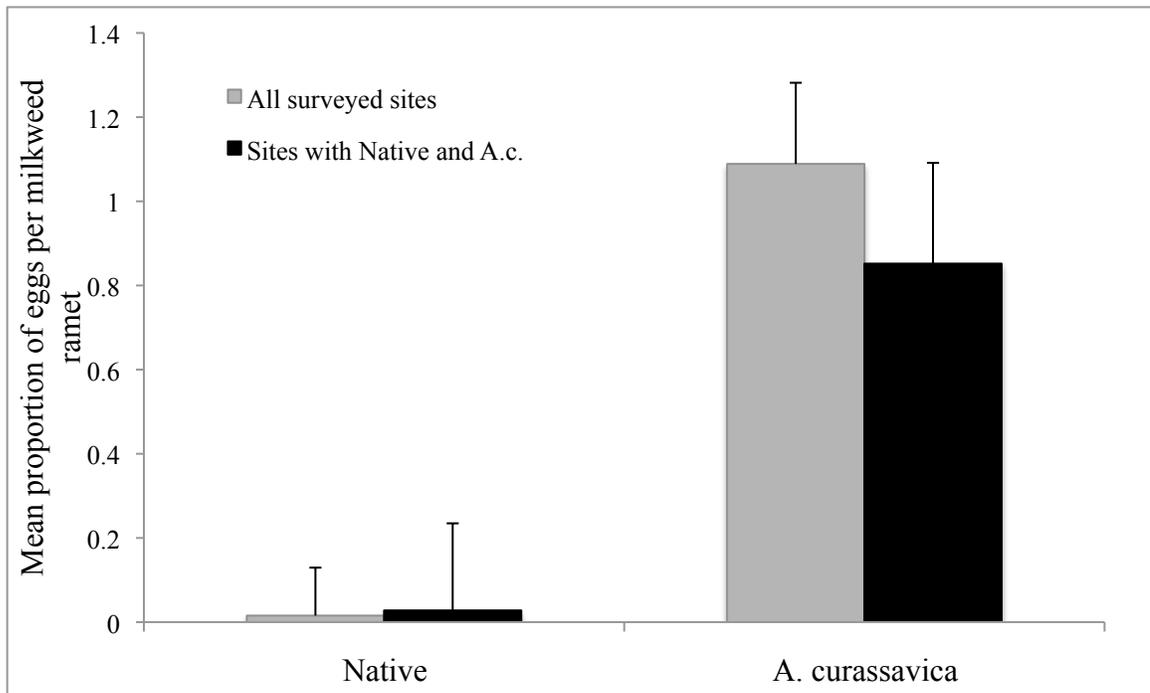


Figure 3.6: Grey bars indicate mean number of eggs per milkweed ramet across all sites surveyed. ($F_{1, 172} = 22.99$, $p < 0.0001$) Black bars indicate mean number of eggs per milkweed ramet only at surveyed sites that contained both native and *A. curassavica* milkweeds. ($F_{1, 61} = 6.77$, $p = 0.01$) Error bars are standard error.

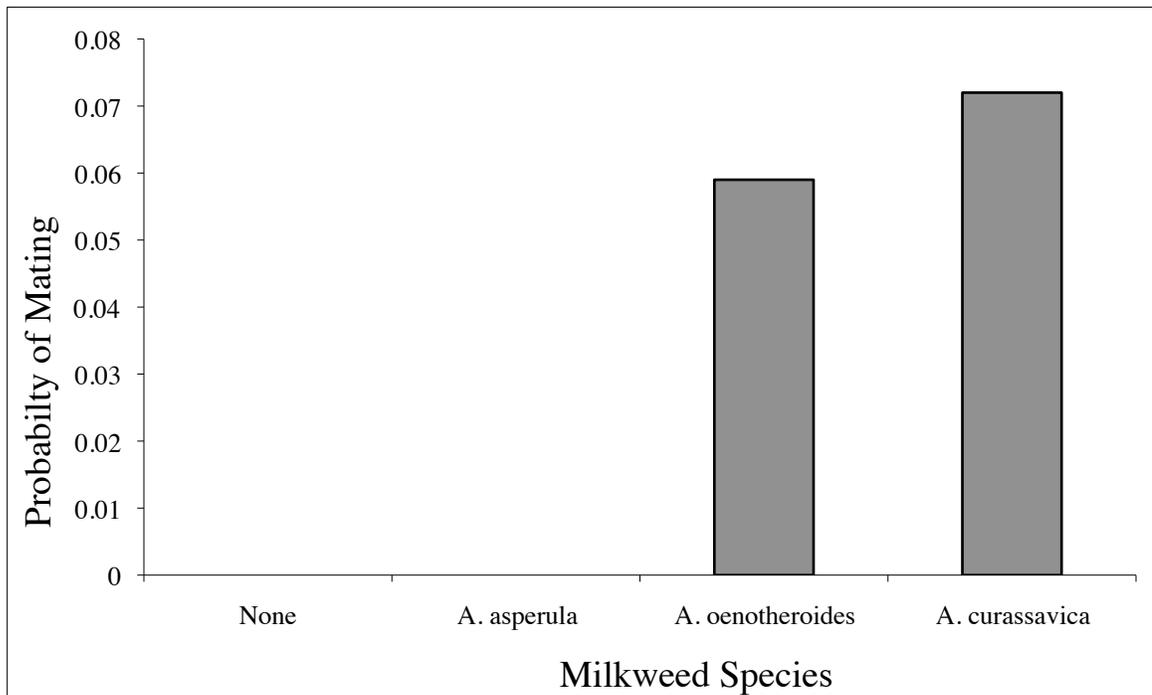


Figure 3.7: Probability of mating by species of milkweed in the cage. Pearson chi-square = 17.06, d.f. = 3, 581, $p = 0.0007$. Error bars are binomial confidence intervals.

COMPREHENSIVE BIBLIOGRAPHY

- Ackery, P. R. and R. I. Vane-Wright. 1984. *Milkweed butterflies: Their cladistics and biology*. Ithaca: Cornell University Press, Comstock Publishing Associates.
- Alonso-Mejia, A., E. Rendon-Salinas, E. Montesinos-Patino and L. P. Brower. 1997. Use of lipid reserves by monarch butterflies overwintering in Mexico: implications for conservation. *Ecological Applications*. 7: 934-947.
- Altizer, S. M. and K. S. Oberhauser. 2000. Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. *Ecol. Entomol.* 25: 125-39.
- Anderson, R. P., D. Lew, and A. T. Peterson. 2003. Evaluating predictive models of species distributions: criteria for selecting optimal models. *Ecol. Mod.* 162: 211-232.
- Arango, N. V. 1996. *Stabilizing Selection in Migratory Butterflies: A Comparative Study of Queen and Monarch Butterflies*. Masters thesis, Univ. of Florida (Gainesville).
- Araújo, M. B., R. G. Pearson, W. Thuiller, and M. Erhard. 2005. Validation of species-climate impact models under climate change. *Glob. Change Biol.* 11: 1504-1513.
- Araújo, M. B., and A. Guisan. 2006. Five (or so) challenges for species distribution modelling. *J. Biogeogr.* 33: 1677-1688.
- Araújo, M. B., W. Thuiller, and R. G. Pearson. 2006. Climate warming and the decline of amphibians and reptiles in Europe. *J. Biogeogr.* 33: 1712-1728.
- Batalden, R., K. Oberhauser and A. T. Peterson. 2007. Ecological niches in sequential generations of eastern North American monarch butterflies (Lepidoptera: Danaidae): the ecology of migration and likely climate change implications. *Env. Ent.* 36: 1365-1373.
- Brower, L. P. 1985. New perspectives on the migration biology of the monarch butterfly, *Danaus plexippus* L. in M. A. Rankin (ed.), *Migration: Mechanisms and Adaptive Significance*. Contrib. Univ. Texas Marine Science.
- Brower, L. P. 1995. Understanding and misunderstanding the migration of the monarch butterfly (Nymphalidae) in North America: 1857-1995. *J. Lepid. Soc.* 49:304-85.
- Brower, L. P., D. R. Kust, E. Rendon-Salinas, E. G. Serrano, K. R. Kust, J. Miller, C. Fernandez del Ray, and K. Pape. Catastrophic Winter Storm Mortality of Monarch Butterflies in Mexico in January 2002. in Oberhauser, K. S. and M. J.

- Solensky. 2004. Monarch Butterfly Biology and Conservation. Cornell University Press.
- Calvert, W. H. 1999. Patterns in the spatial and temporal use of Texas milkweeds (Asclepiadaceae) by the monarch butterfly (*Danaus plexippus* L.) during fall, 1996. J. Lep. Soc. 53: 37-44.
- Clarke, A. R. and M. P. Zalucki. 2004. Monarchs in Australia: on the winds of a storm? Bio. Inv. 6: 123-127.
- Carson, D. J. 1999. Climate modelling: achievements and prospects. QJR Meteorol. Soc. 125: 1-27.
- Crowley, T. J. 2000. Causes of climate change over the past 1000 years. Science 289: 270-277.
- Crozier, L. G. 2004. Field transplants reveal summer constraints on a butterfly range expansion. Oecologia. 141: 148-157.
- DeAnda, A. 2010. The effect and importance of natural enemies on immature monarch (*Danaus plexippus*) populations. Masters thesis, Univ. of Minnesota (Twin Cities).
- Dingle, H., W.A. Rochester and M.P. Zalucki. 2000. Relationships among climate, latitude and migration: Australian butterflies are not temperate-zone birds. Oecologia 124:196-207.
- Forister M. L., Shapiro A. M. 2003. Climatic trends and advancing spring flight of butterflies in lowland California. Glob. Change Biol. 9: 1130–35.
- Goehring, L. and K. S. Oberhauser. 2002. Effects of photoperiod, temperature, and host plant age on induction of reproductive diapause and development time in *Danaus plexippus*. Ecol. Ent. 27: 674-685.
- Goehring, L. and K. S. Oberhauser. 2004. Environmental factors influencing postdiapause reproductive development in monarch butterflies. in K. S. Oberhauser and M. J. Solensky (eds.), Monarch Butterfly Biology and Conservation. Cornell University Press, Ithaca, NY.
- Guisan, A., and U. Hofer. 2003. Predicting reptile distributions at the mesoscale: relation to climate and topography. J. Biogeogr. 30: 1233-1243.
- Hartl FU. 1996. Molecular chaperones in cellular protein folding. *Nature* 381: 571–80.

- Herman, W. S. 1981. Studies on the adult reproductive diapause of the monarch butterfly, *Danaus plexippus*. Biol. Bull. 160:89-106.
- Herman, W. S. and M. Tatar. 2001. Juvenile hormone regulation of longevity in the migratory monarch butterfly. Proc. R. Soc. Lond. B 268: 2509-2514.
- Hill, J. K., C. D. Thomas, and B. Huntley. 2003. Modelling present and potential future ranges of European butterflies using climate response surfaces, pp. 149-167. In C. L. Boggs, W. B. Watt, and P. R. Ehrlich (eds.), *Butterflies Ecology and Evolution Taking Flight*. University of Chicago Press, Chicago, IL.
- Holt, R. D. 1990. The microevolutionary consequences of climate change. Trends Ecol. Evol 5: 311-315.
- Howard, E. and A. K. Davis. 2009. The fall migration flyways of monarch butterflies in eastern North America revealed by citizen scientists. J. Insect Conserv. 13: 279-286.
- Howard, E., H. Aschen, A. K. Davis. 2010. Citizen science observations of monarch butterfly overwintering in the southern United States. Psyche. Vol. 10, Article ID 689301, 6 pages.
- Huey RB. 1991. Physiological consequences of habitat selection. Am. Nat. 137: S91–115.
- Intergovernmental Panel on Climate Change. 1996. in *Climate Change 1995, Impacts, Adaption and Mitigation of Climate Change: Scientific-Technical Analyses*. Cambridge Univ. Press, Cambridge U.K. pp. 171-189.
- IPCC. 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- James, D. G. 1982. Ovarian dormancy in *Danaus plexippus* (L.) (Lepidoptera Nymphalidae)—oligopause not diapause. J. Aust. Entomol. Soc. 21: 31-35.
- James, D.G. 1993. Migration biology of monarchs in Australia. in *Biology and conservation of the monarch butterfly*, eds. Malcolm, S. B. and M. P. Zalucki (Natural History Museum of Los Angeles County, Los Angeles, CA), pp. 189-200.

- Joseph, L. and D. R. B. Stockwell. 2000. Temperature based models of the migration of Swainson's flycatcher *Myiarchus swainsoni* across South America: a new use for museum specimens of migratory birds. *Proc. Acad. Nat. Sci. Philad.* 150: 293-300.
- Journey North: A Global Study of Wildlife Migration. 2011.
<http://www.learner.org/jnorth/>.
- Kammer, A. E. 1971. Influence of acclimation temperature on the shivering behavior of the butterfly *Danaus plexippus* (L.). *Zeitschrift Fur Vergleichende Physiologie* 72:364-369.
- Lindquist S. 1986. The heat-shock response. *Annu. Rev. Biochem.* 55: 1151-91
- Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1987. Monarch butterfly voltinism: effects of temperature constraints at different latitudes. *Oikos.* 49: 77-82.
- Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1993. Spring recolonization of eastern North America by the monarch butterfly: successive brood or single sweep migration?, pp. 253-267. In S. B. Malcolm and M. P. Zalucki (eds.), *Biology and Conservation of the Monarch Butterfly*. Publications of the Los Angeles County Museum of Natural History, Los Angeles, CA.
- Martínez-Meyer, E. 2002. Evolutionary Trends in Ecological Niches of Species. Ph.D. dissertation, University of Kansas, Lawrence.
- Martínez-Meyer, E., A. T. Peterson, and A. G. Navarro-Sigüenza. 2004. Evolution of seasonal ecological niches in the Passerina buntings (Aves: Cardinalidae). *Proc. R. Soc. Lond. B.* 271: 1151-1157.
- Masters, A. R., S. B. Malcolm and L. P. Brower. 1988. Monarch butterfly (*Danaus plexippus*) thermoregulatory behavior and adaptations for overwintering in Mexico. *Ecology* 69: 458-467.
- Masters, A. R. 1993. Temperature and thermoregulation in the monarch butterfly. in S. B. Malcolm and M. P. Zalucki (eds.), *Biology and Conservation of the Monarch Butterfly*. Natural History Museum of Los Angeles County, Los Angeles, California.
- Monarch Larva Monitoring Project. 2011. www.mlmp.org.
- Nakazawa, Y., A. T. Peterson, E. Martínez-Meyer, and A. G. Navarro-Sigüenza. 2004. Seasonal niches of neararctic-neotropical migratory birds: implications for the evolution of migration. *Auk.* 121: 610-618.

- New, M., M. Hulme and P. Jones. 1997. A 1961-1990 Mean Monthly Climatology of Global Land Areas. Norwich, U.K. Climatic Research Unit, University of East Anglia.
- Oberhauser, K. S. 1997. Fecundity, Lifespan and Egg Mass in Butterflies: Effects of male-derived nutrients and females size. *Func. Ecol.* 11: 166-175.
- Oberhauser, K. S., M. D. Prysby, H. R. Mattila, D. E. Stanley-Horn, M. K. Sears, G. Dively, E. Olson, J. M. Pleasants, W. F. Lam and R. L. Hellmich. 2001. Temporal and spatial overlap between monarch larvae and corn pollen. *Proc. Natl. Acad. Sci.* 98: 11913-11918.
- Oberhauser, K. and A. T. Peterson. 2003. Modeling current and future potential wintering distributions of eastern North American monarch butterflies. *Proc. Natl. Acad. Sci.* 100:14063-14068.
- Office of Texas State Climatologist, Texas A&M University. <http://atmo.tamu.edu/osc/>.
- Parmesan C. 1996. Climate and species' range. *Nature* 382: 765–66.
- Parmesan, C., N. Ryrholm, C. Stefanescu, J. K. Hill, C. D. Thomas, H. Descimon, B. Huntley, L. Kaila, J. Kullberg, T. Tammaru, et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*. 399: 579-583.
- Parmesan C. 2003. Butterflies as bio-indicators of climate change impacts. In *Evolution and Ecology Taking Flight: Butterflies as Model Systems*, ed. C. L. Boggs, W. B. Watt, P. R. Ehrlich, pp. 541–60. Chicago: Univ. Chicago Press.
- Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37-42.
- Pearson, R. G., and T. P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecol. Biogeogr.* 12: 361-371.
- Pearson, R. G., C. Raxworthy, M. Nakamura, and A. T. Peterson. 2006. Predicting species' distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *J. Biogeogr.* 34: 102-117.
- Peñuelas, J., I. Filella, and P. Comas. 2002. Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. *Global Change Biology* 8: 531-544.

- Perez, S. M. and O. R. Taylor. 2004. Monarch butterflies' migratory behavior persists despite changes in environmental conditions, pp. 85-88. in K. S. Oberhauser and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.
- Peterson, A.T. and K. C. Cohoon. 1999. Sensitivity of distributional prediction algorithms to geographic data completeness. *Ecol. Mod.* 117: 159-164.
- Peterson, A. T., J. Soberon, and V. Sanchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. *Science*. 285: 1265-1267.
- Peterson, A. T. 2001. Predicting species' geographic distributions based on ecological niche modeling. *Condor*. 103: 599-605.
- Peterson, A. T., V. Sánchez-Cordero, J. Soberón, J. Bartley, R. H. Buddemeier and A. G. Navarro-Sigüenza. 2001. Effects of global climate change on geographic distributions of Mexican Cracidae. *Ecol. Mod.*. 144: 21-30.
- Peterson, A. T. and D. A. Vieglais. 2001. Predicting species invasions using ecological niche modeling: new approaches from bioinformatics attack a pressing problem. *BioScience* 51: 363-371.
- Peterson, A. T., M. A. Ortega-Huerta, J. Bartley, V. Sánchez-Cordero, J. Soberón, R. H. Buddemeier, D. R. B. Stockwell. 2002. Future projections for Mexican faunas under global climate change scenarios. *Nature*. 416: 626-629.
- Peterson, A. T. 2003. Projected climate change effects on Rocky Mountain and Great Plains birds: generalities of biodiversity consequences. *Glob. Change Biol.* 9: 647-655.
- Peterson, A. T., C. Martínez-Campos, Y. Nakazawa, and E. Martínez-Meyer. 2005. Time-specific ecological niche modeling predicts spatial dynamics of vector insects and human dengue cases. *Trans. R. Soc. Trop. Med. Hyg.* 99: 647-655.
- Prysby, M. D. and K. Oberhauser. 2004. Temporal and Geographic Variation in Monarch Densities: citizen scientists document monarch population patterns, pp. 9-20. In K. S. Oberhauser and M. J. Solensky (eds.), *Monarch Butterfly Biology and Conservation*. Cornell University Press, Ithaca, NY.
- Roy D. B., T. H. Sparks. 2000. Phenology of British butterflies and climate change. *Glob. Change Biol.* 6: 407-416.
- Soberón, J. and A. T. Peterson. 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. *Biodiversity Informatics*. 2: 1-10.

- Solensky, M. J. and K. S. Oberhauser. 2009. Male monarch butterflies, *Danaus plexippus*, adjust ejaculates in response to intensity of sperm competition. *Anim. Beh.* 77: 465-472.
- Stockwell, D. R. B. 1992. Machine learning and the problem of prediction and explanation in ecological modeling. Ph.D. dissertation, Australian National University, Canberra, Australia.
- Stockwell, D. R. B. 1999. Genetic algorithms II, pp. 123-144. in A. H. Fielding (ed.), *Machine learning methods for ecological applications*. Bluer Academic Publishers, Boston, MA.
- Stockwell, D. R. B. and D. P. Peters. 1999. The GARP modeling system: problems and solutions to automated spatial prediction. *Int. J. Geog. Inform. Sys.* 13: 143-158.
- Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. Ferreira de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. Van Jaarsveld, G. E. Midgely, L. Miles, M. A. Ortega-Huerta, A. T. Peterson, O. L. Phillips, and S. E. Williams. 2004. Extinction risk from climate change. *Nature*. 427: 145-148.
- Thuiller, W., D. M. Richardson, P. Pysek, G. F. Midgely, G. O. Hughes, and M. Rouget. 2005. Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. *Glob. Change Biol.* 11: 2234-2250.
- Thuiller, W., G. F. Midgely, G. O. Hughes, B. Bomhard, G. Drew, M. C. Rutherford, and F. I. Woodward. 2006. Endemic species and ecosystem sensitivity to climate change in Namibia. *Glob. Change Biol.* 12: 759-776.
- Urquart, F. A. 1987. *The Monarch Butterfly: International Traveler*. Nelson-Hall, Chicago, IL.
- Van Hook, T. 1996. *Monarch Butterfly Mating Ecology at a Mexican Overwintering Site: Proximate causes of non-random mating*. Ph.D. thesis, Univ. of Florida (Gainesville).
- Vane-Wright, R. I. 1991. The Columbus hypothesis: an explanation for the dramatic 19th century range expansion of the monarch butterfly. in S. B. Malcolm and M. P. Zalucki (eds.), *Biology and Conservation of the Monarch Butterfly*. Natural History Museum of Los Angeles County, CA.
- Wiens, J. J. and C. H. Graham. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu. Rev. Ecol. Syst.* 36: 519-539.

- Woodson, R. E. 1954. The North American species of *Asclepias*. Ann. Mo. Bot. Gard.
- York, H. A. and K. S. Oberhauser. 2002. Effects of duration and timing of heat stress on Monarch Butterfly (*Danaus plexippus*) (Lepidoptera: Nymphalidae) development. J. Kans. Entomol. Soc. 75: 290-298.
- Zalucki, M. P. 1981. The effects of age and weather on egg laying in *Danaus plexippus* L. (Lepidoptera: Danaidae). Res. Popul. Ecol. 23: 318-327.
- Zalucki, M. P. 1982. Temperature and rate of development in *Danaus plexippus* L. and *D. chrysippus* L. (Lepidoptera: Nymphalidae) J. Aust. Entomol. Soc. 21: 241-46.
- Zalucki, M. P. and R. L. Kitching. 1982. Temporal and spatial variation of mortality in field populations of *Danaus plexippus* L. and *D. chrysippus* L. larvae (Lepidoptera: Nymphalidae) Oecologia 53: 201-207.
- Zalucki, M. P. and A. R. Clarke. 2004. Monarchs across the Pacific: the Columbus hypothesis revisited. Bio. Jour. Linn. Soc. 82: 111-121.
- Zalucki, M. P. and W. A. Rochester. 1999. Estimating the effect of climate on the distribution and abundance of the monarch butterfly, *Danaus plexippus* (L.): a tale of two continents, pp. 151-163. In J. Hoth, L. Merino, K. Oberhauser, I. Pisanty, S. Price, and T. Wilkinson (eds.), The 1997 North American Conference on the Monarch Butterfly. Commission for Environmental Cooperation, Montreal, Canada.
- Zalucki, M. P. and W. A. Rochester. 2004. Spatial and temporal population dynamics of monarchs down-under: lessons for North America, pp. 219-228. In K. Oberhauser, and M. J. Solensky (eds.), Monarch Butterfly Biology and Conservation. Cornell University Press, Ithaca, NY.
- Zhu, H., R. J. Gegear, A. Casselman, S. Kanginakudru and S. M. Reppert. 2009. Defining behavioral and molecular differences between summer and migratory monarch butterflies. BMC Bio 7: 14.