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Insect eggs protected from high temperatures by limited homeothermy of plant leaves

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SUMMARY

Virtually all aspects of insect biology are affected by body temperature, and many taxa have evolved sophisticated temperature-control mechanisms. All insects, however, begin life as eggs and lack the ability to thermoregulate. Eggs laid on leaves experience a thermal environment, and thus a body temperature, that is strongly influenced by the leaves themselves. Because plants can maintain leaf temperatures that differ from ambient, e.g. by evapotranspiration, plant hosts may protect eggs from extreme ambient temperatures. We examined the degree to which leaves buffer ambient thermal variation and whether that buffering benefits leaf-associated insect eggs. In particular, we: (1) measured temperature variation at oviposition sites in the field, (2) manipulated temperatures in the laboratory to determine the effect of different thermal conditions on embryo development time and survival, and (3) tested embryonic metabolic rates over increasing temperatures. Our results show that *Datura wrightii* leaves buffer *Manduca sexta* eggs from fatally high ambient temperatures in the southwestern USA. Moreover, small differences in temperature profiles among leaves can cause large variation in egg metabolic rate and development time. Specifically, large leaves were hotter than small leaves during the day, reaching temperatures that are stressfully high for eggs. This study provides the first mechanistic demonstration of how this type of leaf-constructed thermal refuge interacts with egg physiology.

Key words: microclimate, respirometry, leaf size, critical thermal maximum, *Manduca sexta*, *Datura wrightii*.

INTRODUCTION

Virtually all aspects of insect biology, from enzyme activity to behavior, are affected by body temperature, and many taxa have evolved sophisticated temperature-control mechanisms. In general, the difficulty of temperature control depends on two factors – body size and mobility. Large size confers thermal inertia, such that large-bodied insects can maintain body temperatures differing from ambient temperatures for longer. Mobility confers the means to move among thermal microhabitats. Indeed, insect behavioral thermoregulation is integral to much of insect physiological ecology (Chown and Nicolson, 2004; Heinrich, 1993; May, 1979).

All insects, however, go through a developmental stage – the egg stage – in which they possess neither large size nor mobility. Because eggs are small, and therefore nearly always isothermal with nearby microenvironments, embryo temperature is determined by maternally chosen microhabitats. Here, we focus on phytophagous insects that oviposit, as most do, directly onto host plants. For these eggs, microsite experience reflects interactions between ambient environmental characteristics (e.g. air temperature, solar radiation, wind speed) and leaf morphology and physiology (e.g. leaf size, color, pubescence, transpiration). Eggs lie in boundary layers of relatively still air, which resist heat and moisture transfer between a leaf and its surroundings. When boundary layer conductance is low – e.g. big leaves in calm wind – large differences between leaf and air temperatures can occur (Martin et al., 1999; Monteith and Unsworth, 1990; Nobel, 2005; Schuepp, 1993). Conversely, when conductance is high, transpiration can depress leaf temperature below ambient. This raises the possibility that insect eggs, due to small size and proximity, exploit the physiology of their host leaves to avoid damagingly high temperatures.

Testing this idea requires evaluating both leaf and egg physiology. Unlike other life stages, which have the advantage of mobility and functioning organs, embryos rely solely on cellular mechanisms – particularly heat shock proteins – to survive thermal stress (Feder and Hofmann, 1999; Hamdoun and Epel, 2007; Kultz, 2005). Because expression of cellular mechanisms depends on developmental stage, precise matching between eggs and their expected environment may be impossible (Heikkila, 2003; Krebs and Feder, 1998). Alternatively, selection may favor strategies that allow embryos to be robust to extremes, such as maternal pre-loading of heat shock protein (HSP) (e.g. Feder, 1997; Feder et al., 1997; Roberts and Feder, 2000). Indeed, while eggs have been viewed historically as vulnerable to high temperatures, development is generally successful under real-world conditions (Hamdoun and Epel, 2007).

The second, complementary issue is leaf microclimate. Do leaf microclimates buffer ambient thermal variation, such that eggs on leaves rarely experience extremes? Mahan and Upchurch argue that plants are ‘limited homeotherms’, i.e. that plants control transpiration to optimize temperature for cell function (Mahon and Upchurch, 1988). Because photosynthesis, growth and reproduction rely on enzymes with species-specific optimal temperatures, plants that can lower their leaf temperatures may avoid the cost of shutting down these processes in the heat of the day (Hatfield and Burke, 1991; Zangerl, 1978). While a leaf’s minimum temperature is environmentally controlled, its high temperature is subject to greater control by the leaf itself. This control stems from stomatal behavior.

Open stomata allow for increased transpiration and evaporative cooling, particularly when the vapor pressure difference between leaf and air is high. Alternatively, stomatal behavior may act to limit water loss; leaves with closed stomata may be warmer than air temperature due to solar heating (Field et al., 1982). In the same way that insect heat balance depends on size, so does leaf heat balance. Because leaf size affects boundary layer thickness, leaves even within the same plant may vary in their ability to thermoregulate and thus provide different microhabitats.

We examined the degree to which leaves buffer ambient thermal variation and whether that buffering benefits leaf-associated insect eggs; in particular, whether buffering protects eggs from fatally high ambient temperatures and whether leaf thermal microhabitats support rapid development. We used eggs of the sphinx moth, *Manduca sexta* L. (Lepidoptera: Sphingidae) and its primary host in southern Arizona, *Datura wrightii* Regel (Solanaceae), a broad-leafed perennial plant. *M. sexta* occurs across much of North America south of ~42°N, extending to southern South America. Females oviposit exclusively on Solanaceae (Madden and Chamberlin, 1945; Yamamoto and Fraenkel, 1960) and one species of Martyniaceae (Mechaber and Hildebrand, 2000). Females lay an average of ~300 eggs, each deposited individually under the host plant leaves (Madden and Chamberlin, 1945). *M. sexta* has become a model insect in biology, yet has rarely been studied in its natural habitat. In a laboratory population, *M. sexta* eggs are oxygen-limited above 32°C (Woods and Hill, 2004); since air temperatures in Arizona regularly reach 45°C, *Datura* leaves may provide critical microclimates. In this study, we: (1) measured temperature variation at oviposition sites in the field, (2) manipulated temperatures in the laboratory to determine the effect of different thermal conditions on embryo development time and survival, and (3) tested embryonic metabolic rates, measured as rates of CO_2 emission, over increasing temperatures. Collectively, our results suggest that *M. sexta* eggs in southern Arizona often experience temperatures that are stressfully or fatally high. Moreover, even moderate temperatures caused large differences in embryo metabolic rate and development time. Even slight reduction in leaf temperatures moves eggs away from critical thermal maxima, suggesting that eggs are protected by the limited homeothermy of their hosts.

MATERIALS AND METHODS

Animals

We used wild *Manduca sexta* L. (Lepidoptera: Sphingidae) collected as eggs and larvae from a common host, *Datura wrightii* Regel (Solanaceae), around Tucson, AZ, USA in July and August 2008. In Arizona, *M. sexta* is active during the monsoon season from July to September (Riffell et al., 2008). Females attach eggs singly to the lower host leaf surface; eggs hatch after ~4 days, and larvae typically develop through 5 or 6 instars (Kingsolver, 2007). For this study, we reared ~200 individuals on cut leaves of *D. wrightii* and allowed them to pupate in soil. Moths emerged in a 2×2×2 m flight cage and had access to potted *D. wrightii* and *Nicotiana tabacum* as oviposition substrates and to sugar water and *Datura* flowers as food sources. Eggs collected from these moths were used for all experiments.

Leaf temperatures

The primary host plant for *M. sexta* in southwestern USA is *D. wrightii*, a broad-leaf, perennial herb. In September 2007, we attached twenty-four 40-gauge, copper–constantan thermocouples (constructed by K.P.) to the undersides of leaves on four *D. wrightii* plants growing in a field at the University of Arizona Campus Agricultural Center in Tucson, AZ. Twelve pairs of adjacent leaves, one small (4–5 cm in length) and one large (10–12 cm), with otherwise similar height, orientation, shading and color, were selected. These size classes are within the range of leaves on which females normally oviposit (K.P., unpublished). All pairs were on the exterior of the plant, where *M. sexta* primarily oviposits, which minimizes self-shading during the day. Thermocouples were attached 1 cm from the leaf edge, a typical oviposition location for *M. sexta* eggs (mean ± s.e.m.=0.92±0.06 cm from edge, N=40). Previous measurements by Potter and others (e.g. Chappell, 1983) show that in desert environments there are steep thermal gradients from ground level up to 10–20 cm. Leaf pairs were therefore selected at a range of heights above 20 cm (leaves, 29–96 cm; plants, 67–105 cm). One additional thermocouple, used to measure ambient air temperature, was placed under a radiation shield and fixed 50 cm above the ground. The mean height of the thermocouples was not significantly different from the height of the ambient air probe (t=–0.5084, P=0.62, N=9). Temperatures were sampled every minute, averaged over 10-min blocks, and recorded to a datalogger (CR10X; Campbell Scientific Inc., Logan, UT, USA) fitted with a thermocouple multiplexer (AM25T; Campbell Scientific Inc.). Thermocouples were checked daily, and any that had become detached from the leaf surface were removed from further analysis. Nine pairs of thermocouples (18 total) remained for analysis.

Temperature, survival and development rate of eggs

Multiple moths were allowed to oviposit for 3 h (21.30–00.30 h). Eggs were immediately collected, and a random subset placed into an aluminum thermal gradient bar (for details, see Woods and Bonnecaze, 2006). Temperature gradients in the bar were set by circulating controlled-temperature water through chambers in each end. Down the length of the bar were 12 evenly spaced rows of eight wells each, into which we placed 96 eggs (mean ± s.e.m.=2.03±0.02 mg). Copper–constantan thermocouples were also placed in each row, and temperature was logged every 10 min. Bath temperatures were set to mimic the daily temperature cycles we measured on *D. wrightii* leaves in Tucson (Fig. 1). During the night, all rows were held at 24°C. During the day, temperatures were ramped up slowly such that each row reached peak temperature, ranging from 29.5°C to 46°C (1.5°C intervals between each adjacent row), for 3 h and then ramped back down (see Fig. 3A). Time-lapse movies (30 frames h^{-1}) of the bar, visualized from above, were taken using webcams and CamPanel monitoring software (Eagletron, v. 2.6.6; www.trackercam.com/TCamWeb), allowing us to determine hatching time of each egg. Eggs not hatched after 7 days were observed under a microscope and scored as fertile (dead embryo inside) or infertile (no embryo inside). Infertile eggs were removed from analysis.

Effects of heat shock on egg survival

Eggs were collected after one night of oviposition and kept at room temperature (~24°C). Three days later, 96 eggs were heat shocked for two hours. Eggs were dropped simultaneously into a thermal gradient bar (described above), which varied in temperature from 37°C to 48°C along its length, with a 1°C difference between rows (N=8 eggs per temperature; 12 temperatures). After two hours, the eggs were removed from the bar and returned to room temperature for the remainder of development. Seven days after oviposition, eggs were scored for hatching success. Unhatched eggs were observed under a microscope to determine whether they were infertile or contained partly developed embryos; infertile eggs were removed from analysis.

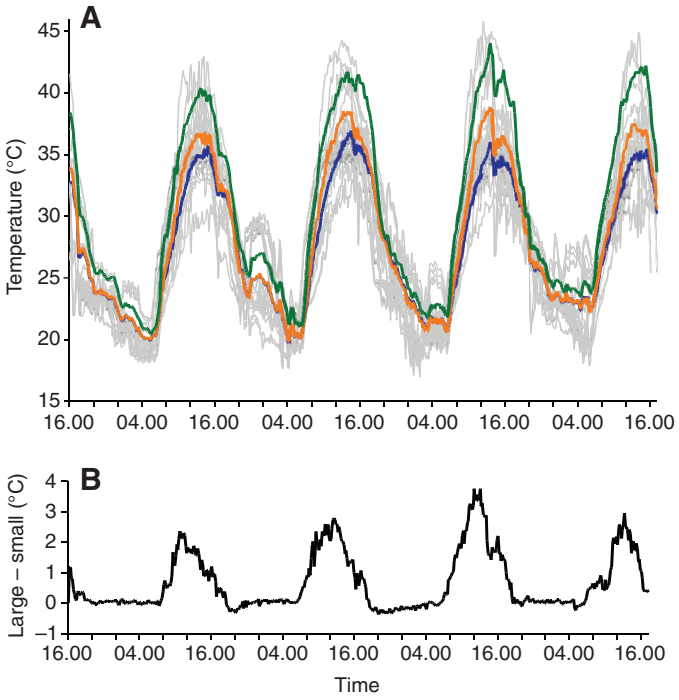

Fig. 1. (A) Gray traces (N=18) show temperatures under *Datura wrightii* leaves in Tucson, AZ, USA (Sept. 2007). The green line is ambient temperature, measured 50 cm above the ground. Orange and blue lines represent mean temperature for large and small leaves, respectively (N=9 each). Leaves were selected as pairs of adjacent leaves, one small (4–5 cm in length) and one large (10–12 cm), as equal as possible in other measurements (e.g. height, orientation, shading, color). Temperature measurements were taken every minute and averaged over 10-min blocks. (B) Mean difference between adjacent pairs of small and large leaves (N=9).

Egg respiration as a function of temperature

Carbon dioxide emission was measured using flow-through respirometry. Eggs laid overnight were collected in the morning and split into two batches of 10–15 eggs each: one batch was measured on day one (the day following the night of oviposition), and the second on day three. Four paired trials were run (N=8 total). Egg batches were weighed and placed into a water-jacketed stainless-steel chamber (for details, see Woods and Hill, 2004). Chamber temperature was controlled by water from a recirculating bath. Eggs were allowed to equilibrate under experimental conditions in the water-jacketed chamber for at least 20 min before temperature ramps began. Bath temperature started at 27°C and was increased by 2°C every 10–12 min until chamber temperature exceeded 50°C. Dry, CO_2-free compressed air was directed past eggs at a rate of 30 ml min^{-1} (STPD) and then into the CO_2 analyzer (Licor LI-7000; Lincoln, NE, USA). All gases were handled using Bev-a-line IV tubing (Cole Parmer, Vernon Hills, IL, USA). The analyzer was calibrated regularly with pure N_2 and 100 p.p.m. CO_2 in N_2 (Airgas). Data were logged using ExpeData software (v. 0.2.48; Sable Systems, Las Vegas, NV, USA), receiving digital signals from an A/D converter (UI2, Sable Systems), which itself received analog signals from the instrument. For a control, we logged temperature in a separate chamber otherwise identical to the experimental chamber except that it was fitted with a T-type thermocouple (connected to a TC-1000 thermocouple meter; Sable Systems) that extended into the chamber's air space. Mass-specific CO_2 emission was calculated from concentration, flow rate and batch mass.

RESULTS

Leaf temperatures

In Arizona, temperatures under *D. wrightii* leaves varied substantially, suggesting that *M. sexta* eggs are exposed to a wide range of temperatures. Individual leaves exhibited diurnal fluctuations of more than 20°C (Fig. 1A), with peak daytime temperatures ranging from 33 to 46°C (gray traces, Fig. 1A). There was no relationship between thermocouple height and maximum daily temperature of either small (R^2=0.26, P=0.16, N=9) or large (R^2=0.09, P=0.42, N=9) leaves. Most of the time, most leaves were cooler than the ambient air. At night, leaf and air temperatures were similar, and leaf-to-leaf differences were smaller. During the day, large leaves were consistently warmer than their matched small leaves (Fig. 1B). Large leaves had daytime high temperatures that were, on average, 3.0°C warmer than those of small leaves (paired t-test, N=9 pairs, P=0.012). Minimum nighttime temperatures, by contrast, were indistinguishable between sizes (paired t-test, N=9 pairs, P=0.45). Functionally, leaves of *D. wrightii* showed size-dependent limited homeothermy. In both size classes, leaf temperature rose with ambient temperature. But for small leaves, the relationship appeared to be curvilinear, deviating more at higher ambient temperatures. We confirmed this impression by fitting a second-order model ($T_{leaf} \sim size \times T_{air} + size \times T_{air}^2$). The main second-order term was not significant, but there was a significant interaction between it and size (Table 1). Importantly, *D. wrightii* functions as a limited homeotherm, primarily due to the cooling of small leaves (Fig. 2). Small leaves show a non-linear relationship between air and leaf temperature (ANOVA, P=0.020, N=9), whereas large leaves do not (ANOVA, P=0.61). Each point in Fig. 2 represents an hourly average of leaf temperatures *vs* hourly average air temperature over the course of the experiment. Leaf buffering helps maintain temperatures within a 'safe' range for eggs – the dotted line at 40°C represents the maximum daily temperature at which 100% of eggs survived (see below).

Temperature, survival and development rate of eggs

Gradient-bar temperatures were cycled to mimic *D. wrightii* leaf temperatures in the field. Although each treatment experienced fluctuating temperatures throughout egg development, we refer to each treatment here by its peak daily temperature, which was held for 3 h each day (Fig. 3A; 12 treatments; N=8 eggs per treatment).

Table 1. ANOVA summary statistics for the effects of leaf size and ambient temperature (T_{air}) on leaf temperature

	d.f.	SS	F	P
All leaves				
Leaf size	1	22.3	51.65	<0.0001
T_{air}	1	5555.4	12851.83	<0.0001
T_{air}^2	1	1.1	2.59	0.109
Leaf size × T_{air}	1	28.5	65.91	<0.0001
Leaf size × T_{air}^2	1	2.1	4.91	0.028
Residuals	188	81.3		
Large leaves				
T_{air}	1	3189.9	10502.57	<0.0001
T_{air}^2	1	0.1	0.2606	0.611
Residuals	94	28.5		
Small leaves				
T_{air}	1	2394.1	4268.97	<0.0001
T_{air}^2	1	3.2	5.637	0.020
Residuals	94	52.7		

Small leaves show a non-linear relationship between air and leaf temperature whereas large leaves do not.

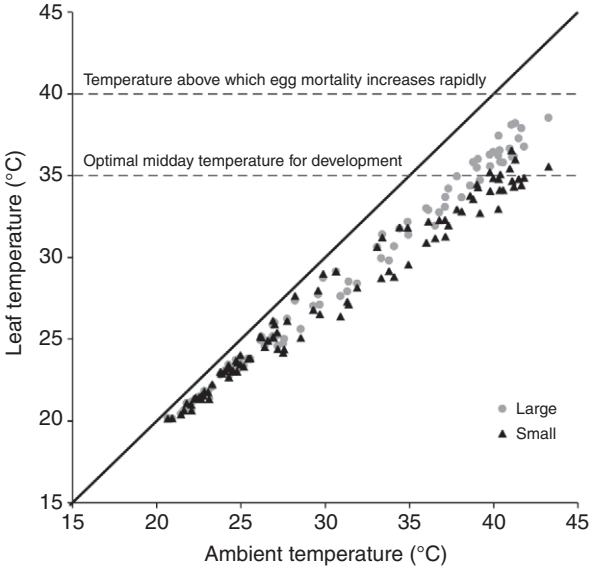

Fig. 2. *Datura wrightii* leaf temperatures *vs* ambient temperature in Tucson, AZ, USA (Sept. 2007). Each point represents an hourly mean of leaf temperatures from nine small leaves (4–5 cm in length; black triangles) or nine large leaves (10–12 cm in length; gray circles) *vs* hourly mean ambient temperature. Broken lines at 35 and 40°C represent the optimal midday temperature for *Manduca sexta* egg development and the threshold daily temperature above which eggs have reduced survival, respectively. Leaf buffering, particularly by small leaves, helps keep eggs within a 'safe' temperature range.

Hatching success was 100% for treatments up to 40°C, 62.5% for 41.5°C, and 0% for 43°C and above (Fig. 3B). Temperature also had a strong effect on time to hatching. Eggs at 35.5°C had the shortest time to hatching (average 90 h), and eggs at warmer and cooler temperatures had longer development times (up to 130 h). The best-fit equation for egg development time was a 2nd-order polynomial (R^2=0.75; Fig. 3C).

Effects of heat shock on egg survival

The hottest 2-h heat shock survived by eggs was 44°C, although only 75% of those eggs (6/8) hatched (Fig. 4). No eggs hatched in the four hottest treatments (45–48°C). Nearly 100% of eggs successfully hatched at heat shocks below 44°C, with the exception of one dead embryo in the 42°C treatment. Logistic regression gave an LD_{50} temperature of 44.11±0.24°C. There were four infertile eggs, which were removed from the analysis, reducing the sample size for the 39, 41 and 43°C treatments to 6, 7 and 7, respectively.

Egg respiration as a function of temperature

As expected, one-day-old eggs had lower metabolic rates than three-day-old eggs (Fig. 5). The plateau phase of carbon dioxide emission ($\dot{V}_{CO_2}$) around 38°C represents the highest sustained metabolic flux rate possible. Following that, $\dot{V}_{CO_2}$ drops rapidly in the 'mortal fall' phase; in adult insects, this stage is followed by cessation of spiracular control and voluntary motor control (Lighton, 2007; Lighton and Turner, 2004). Subsequently, $\dot{V}_{CO_2}$ rises to a 'postmortal peak' [possible causes are discussed by (Lighton and Turner, 2004)]. We analyzed the respiratory data by linear modeling (lm) of natural splines (ns) in R (v. 2.8.1; www.r-project.org). The model call was: lm[$\dot{V}_{CO_2}$~ns(Temperature, d.f.=10)+Age+Age/Batch+ns(Temperature, d.f.=10):Age], where the first term accounts for the overall effects of temperature across egg ages, the second term for the effect of egg age across temperatures, the third for batches within ages, and the fourth for interactions between temperature and age. All terms were highly significant (Table 2), including the interaction between temperature and egg age. This interaction indicates that metabolic traces of one-day-old eggs were significantly right-shifted; i.e. younger eggs were slightly more thermotolerant.

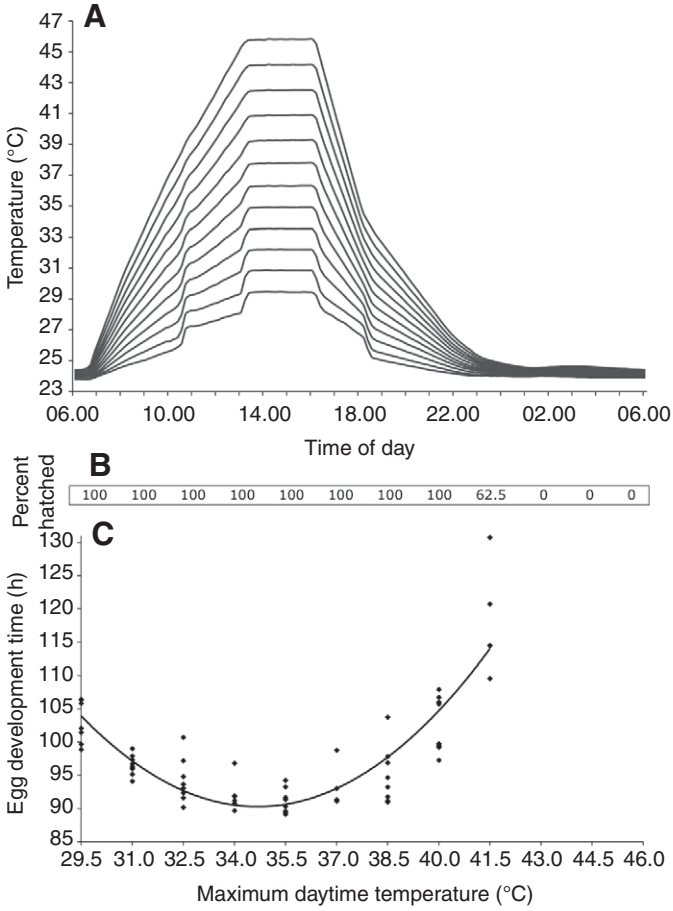

Fig. 3. (A) Twelve temperature treatments for thermal gradient bar experiment. Each line represents one treatment (N=8 eggs each), such that eggs in the coolest treatment experienced fluctuating temperatures with a daily maximum of 29.5°C (bottom line), and eggs in the hottest treatment experienced fluctuating temperatures with a daily peak of 46°C (top line). (B,C) Development time and percent survival for eggs in each of the 12 temperature treatments (labeled by their daily maximum temperature). The curved line shows the best-fit polynomial for egg development time (R^2=0.75; y=0.52x^2–35.4x+703).

DISCUSSION

Our results show that egg survival is facilitated by the limited homeothermy of host plants: *Datura wrightii* leaves buffer *Manduca sexta* eggs from fatally high ambient temperatures in the southwestern USA. Moreover, small differences in temperature profiles among leaves can cause large variation in egg metabolic rate and development time. Many *D. wrightii* leaves – particularly large ones – have daytime temperatures that are stressfully high for eggs.

Egg fitness is a function of both survival and development time. Survival declines dramatically when daytime temperatures exceed 40°C, and the severity of the decline depends on time of exposure. *M. sexta* eggs from a laboratory population held at constant temperatures failed to hatch above 32°C (Woods and Bonnecaze,

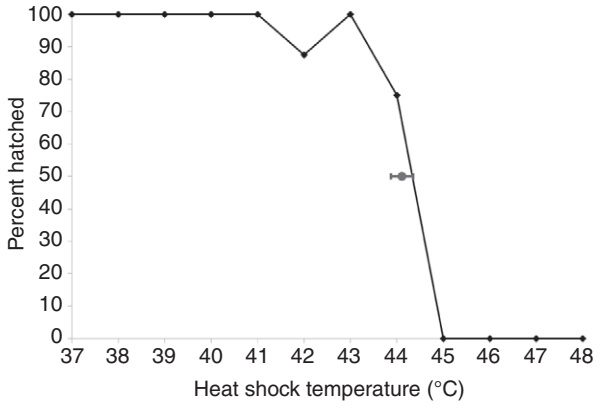

Fig. 4. Percent hatching success for eggs that experienced 2-h heat shocks of different temperatures (*N*=6–8 per treatment). The upper 95% confidence interval (CI) for the data points at 42 and 44°C includes 100%. Eggs were held at 24°C for all of development, with the exception of the heat shock on day 3. The LD_{50} (±95% CI) at 44.11°C is indicated by a gray circle.

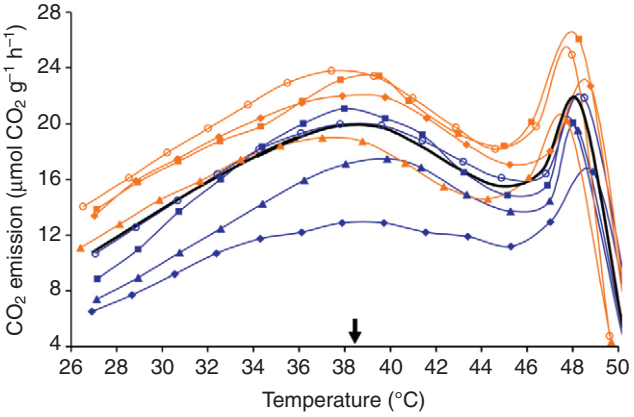

Fig. 5. CO_2 emission by 1- and 3-day-old eggs (blue and orange lines, respectively) over increasing temperatures (2°C every 10–12 min). *N*=8 total traces, 10–15 eggs each. Matching symbols denote paired egg batches, and the black line represents the mean of all traces. Metabolism of older eggs was higher than that of younger eggs, and CO_2 emission of both groups peaked near 38°C (arrow) and declined at higher temperatures.

2006). In our study, while all *M. sexta* eggs survived a single 2-h heat shock of 43°C (Fig. 4), no eggs survived daily 43°C exposure when subjected to cyclical temperatures mimicking those on leaves (Fig. 3). Even for surviving eggs, however, small differences in midday temperature caused large variation in development times (up to 50% longer; range, 3.75–5.5 days). Eggs developed fastest when midday temperatures reached ~35°C (Fig. 3), which corresponds closely to the highest rates of CO_2 emission, around 38°C. Although we did not show it in our experiments, long development times may translate into enormous costs in nature; in *M. sexta*, ~20–45% of mortality on *D. wrightii* occurs in the egg stage from egg predators and parasitoids (Mira and Bernays, 2002). Therefore, even small changes in midday temperature should affect egg interactions with these threats.

Microclimate buffering

Our leaf temperature data support the microclimatic buffering hypothesis; leaf temperatures were usually cooler than ambient, especially during the hottest part of the day. Interestingly, although both size classes protect eggs, larger leaves were consistently warmer during the day than their matched small leaves. Indeed, only small leaves show limited homeothermy as defined by Mahan and Upchurch (Mahan and Upchurch, 1988). This pattern likely reflects differences in the physiology and morphology of smaller *D. wrightii* leaves, which have higher transpiration rates during the day than large leaves (G. Barron-Gafford, unpublished) and higher boundary layer conductance (Jones, 1992). Smith (Smith, 1978) surveyed leaf temperatures at solar noon and found that several large-leaved desert species, including *D. wrightii* (previously *D. metaloides*), had low leaf temperatures coupled with high transpiration rates, in addition to correspondingly low temperatures for maximum photosynthesis. *D. wrightii*'s deep tap root may provide sufficient water for high transpiration even in Arizona's dry climate; indeed, each plant opens as many as 100 nectar-filled (~65 µl) flowers each night (Raguso et al., 2003). In *D. wrightii*, >90% maximum photosynthesis occurs when leaf temperatures are between 27.5 and 35.5°C (Smith, 1978). Our study shows that small leaves can stay within this range nearly all of the time (Fig. 2); large leaves are less able to cool themselves at high temperatures.

Studies of limited homeothermy have focused on the costs of shutting down photosynthesis and cellular function during the day and the potential adaptive value of evaporative cooling for leaves. Our study reveals a counter consideration: a plant's costs of shutting down photosynthesis may be outweighed by the benefit of killing its herbivores' eggs. Herbivory by insects can lower plant reproductive success by reducing both the attractiveness of plants to pollinators and the resources available for nectar, seed and fruit production (Lehtilä and Strauss, 1997; Mothershead and Marquis, 2000). For example, a single *M. sexta* larvae can process up to 3000 cm^2 of leaves (Jones and Thurston, 1970; Madden and Chamberlin, 1945) and can defoliate its host by the time it pupates. The plant strategy of making microclimates less suitable for herbivore development, even at a cost to a plant's own growth, may be favored in areas of heavy herbivory. The consequences of limited homeothermy will be greatest when there are few natural enemies of the eggs. Indeed, some plant species show a 'hypersensitive response' to oviposition. When eggs are deposited onto their leaves, the tissue around the egg rapidly turns necrotic and in some cases falls off (Balbyshev and Lorenzen, 1997; Shapiro and DeVay, 1987). Because it no longer transpires, the necrotic tissue can get hot enough to kill the egg.

Thermal ecology of *M. sexta* eggs

Species persist only when all life stages succeed, so processes in any individual stage may shape a species' overall ecology. For eggs, thermal tolerance may determine latitudinal and altitudinal patterns

Table 2. ANOVA summary of egg metabolic rates

Source	d.f.	SS	*F*	*P*
Spline (temperature, d.f.=10)	10	1786	64.2	<0.0001
Age	1	374	134.5	<0.0001
Batch within age	6	353	21.2	<0.0001
Spline × age	10	120	4.3	<0.0001
Residuals	84	234		

Data were modeled in R (v. 2.8.1) using the natural spline function with 10 degrees of freedom.

of egg performance, overall distribution and abundance of species, and oviposition decisions by females.

Where females choose to oviposit should be under strong selection, as egg microhabitat determines embryo performance. First, there *is* temperature variation among leaves; second, the variation is predictable from leaf size; third, leaf temperatures are high enough to be dangerous under some circumstances. Similar to leaf miners in sun *vs* shade leaves (Pincebourde and Casas, 2006; Pincebourde et al., 2007), *M. sexta* females face a tradeoff between having their eggs develop rapidly and minimizing the chance of overheating. To avoid thermal extremes, females could modify oviposition timing or location; i.e. lay eggs during optimal seasons or in optimal sites. In particular, females should prefer to oviposit on smaller leaves. From a purely thermal perspective, small leaves should maximize the chance of egg survival in hot environments. Finally, while longer development is generally predicted to increase mortality due to predators and parasitoids, how temperature affects egg–parasite interactions is still unknown. If parasitoids have lower thermotolerance than the embryo itself, hotter eggs may have a survival advantage even if development time is extended (Thomas and Blanford, 2003). Alternatively, high temperature may disrupt the transcription of immune genes that help eggs defend against parasitoids (Abdel-latief and Hilker, 2008). Future work will evaluate female oviposition decisions in light of these results.

Implications for insects and climate change

As global temperatures and the frequency of extreme weather events increase (Meehl et al., 2007), changes in insect phenology, physiology, behavior and range are occurring (Parmesan, 1996; Parmesan, 2001; Parmesan, 2006; Walther et al., 2002). In particular, in the northern hemisphere, the northern range of many insects has expanded without a corresponding contraction of the southern border (Parmesan et al., 1999). This implies that conditions in the north are no longer too cool, while conditions in the south have not warmed too much. ‘Limited homeothermy’ of plant leaves may partially explain these results. Specifically, leaf buffering is asymmetrical – it depresses hot temperatures but doesn’t raise low temperatures (Mahan and Upchurch, 1988; Upchurch and Mahan, 1988). Thus, leaves may filter unsuitably hot macroenvironments into still-reasonable microenvironments, providing refuge from increasing ambient temperatures. Our data on thermal interactions between *M. sexta* and *D. wrightii* are the first mechanistic demonstration of how this kind of thermal refuge can interact with egg physiology.

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