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Cross-stage consequences of egg temperature in the insect *Manduca sexta*

Kristen A. Potter[1,*], Goggy Davidowitz[2] and H. Arthur Woods[1]

[1]*Division of Biological Sciences, University of Montana, Missoula, Montana 59812, USA; and* [2]*Department of Entomology, University of Arizona, Tucson, Arizona 85721, USA*

Summary

1. An organism's environment, particularly early in development, can profoundly shape its future phenotypes. While the long-term consequences of embryonic temperature are well studied in vertebrates, insects have complex life cycles that may uncouple temperature's effects in one stage from physiology in the next.

2. This study examines how egg temperature affects insect performance across all subsequent life stages. We focused on the hawkmoth, *Manduca sexta*, and examined how egg temperatures affected hatching time, hatchling mass, larval growth, development time, head capsule size, pupal mass and adult fecundity. Eggs were exposed to either diurnal temperature cycles or a single heat shock; in both experiments, temperatures were within the range to which eggs are typically exposed in the field.

3. Although the consequences of egg temperature varied depending on the type of treatment, both cycling temperatures and heat shock affected egg development time and initial larval growth rate, which likely have fitness consequences for *M. sexta* in nature. In contrast, egg temperature had no persistent effect on any trait measured in later larval stages, pupae or adults.

4. Organisms with complex life cycles – *Manduca* has four distinct life stages and multiple larval sub-stages – may benefit from rapid compensation for poor early conditions. Additionally, the modularity of insect life cycles may help insects cope with environmental variability by insulating later stages from disturbances during embryogenesis.

Key-words: complex life cycles, *Datura wrightii*, embryonic temperature, growth rate, head capsules, heat shock, life-history stages, metamorphosis, phenotypic plasticity

Introduction

Environmental context plays a crucial role in the development of all organisms. Phenotypes emerge from complex interactions between genes and environment (Gilbert & Epel 2008; Monaghan 2008), and generally, the earlier an environmental disturbance, the stronger its long-term effects (Lindström 1999). A poor early environment may prompt compensatory responses later in life (Metcalfe & Monaghan 2001), or it may induce lasting phenotypic change. Here, we focus on phenotypic plasticity induced by embryonic temperature. At an extreme, egg temperature determines life-history trajectory, as in reptiles with temperature-dependent sex determination (Bull 1980). Egg temperature also can affect subsequent growth rate (Hare *et al.* 2009), behaviour (Flores, Tousignant & Crews 1994), muscle phenotype (Macqueen *et al.* 2008), bone morphology (Hammond, Simbi & Stickland 2007), immune function (Ardia, Pérez & Clotfelter 2010) and fecundity (Callebaut 1991).

All of the examples above are from vertebrates in which adults develop incrementally from foetuses. In contrast, insect life cycles are more disjunct, progressing across multiple life stages and (in holometabolous species) culminating in metamorphosis. Life cycle modularity may allow insects to uncouple environmental disturbance in one stage from physiology in the next. For example, in birds, egg temperature directly affects the formation of ovaries (Callebaut 1991); in insects, reproductive organs do not develop until later, typically during metamorphosis.

Furthermore, insects may uniquely benefit from compensatory growth after a poor egg environment. Unlike an organism that grows incrementally, an insect's size at a precise point – metamorphosis – disproportionately influences its future survival and fecundity (Taylor, Anderson & Peckarsky 1998). Rapid compensation after an early stress may eliminate the cost of reaching this key transition at a suboptimal size. Compensation is common in insects, and can occur by

*Correspondence author. E-mail: kristen.potter@mso.umt.edu

changes in the rate or the duration of growth (Simpson & Simpson 1990; Yang & Joern 1994; Woods 1999). Although larval temperature clearly influences development (e.g. Qayyum & Zalucki 1987; Crill, Huey & Gilchrist 1996; Pritchard, Harder & Mutch 1996; Gillooly & Dodson 2000; Steigenga & Fischer 2009), the consequences of *embryonic* environment for later stages have rarely been addressed. Yet, while larvae can thermoregulate in nature (Casey 1976; Capinera, Wiener & Anamosa 1980; Kührt, Samietz & Dorn 2005), eggs are exposed to temperatures that are entirely out of their control.

Egg temperature could affect organismal performance in multiple ways. Here, we organize them into three categories of developmental plasticity that persist for different durations. First, egg temperature may cause long-term shifts in form and function through adulthood. For example, in Atlantic salmon, temperature during embryogenesis alters adult muscle phenotype, including muscle fibre number, fibre diameter and nuclear density (Macqueen *et al*. 2008). Many traits in birds (e.g. Callebaut 1991; Deeming & Ferguson 1991), reptiles (e.g. Bull 1980) and fish (e.g. Johnston 2006; Macqueen *et al*. 2008) illustrate this type of persistent 'legacy' effect. Second, egg temperature may cause shorter term shifts in form and function that appear only in the adjacent, larval, stages. For example, larvae that hatch from warmer eggs may have lower protein : lipid ratios (Geister *et al*. 2009) or up-regulated levels of heat shock proteins. These larval effects are similar in kind to legacy effects, but persist for less of the subsequent life cycle. They also include compensatory responses that mitigate the effects of poor early conditions. In damselflies, e.g. reduced larval size due to a temporary food limitation triggers a subsequent compensatory increase in growth rate, so that initial size differences are resolved by adulthood (Dmitriew & Rowe 2005). Finally, egg temperature may have only embryonic effects – direct effects on embryo survival, hatching mass and time to hatching – but no longer-term effects. Differences in these variables may affect fitness, e.g. by extending development time, even if larvae have the same mass-corrected growth after hatching.

In this study, we asked: what traits does egg temperature affect, and over what time scales do those effects persist? We focused on traits that are typically central to fitness – e.g. size, development time, growth rate and potential fecundity. We used *Manduca sexta* (Lepidoptera: Sphingidae) (Fig. 1), a hawkmoth that ranges over much of North and South America, and is therefore subject to a wide range of temperatures during development. Females lay eggs singly under host plant leaves (Madden & Chamberlin 1945). A previous study examined the temperatures to which *M. sexta* eggs are exposed in southeastern Arizona (Potter, Davidowitz & Woods 2009). Nighttime temperatures are similar leaf to leaf, but peak daytime temperatures of individual leaves span ~15 °C and reach temperatures that are stressfully high for eggs. Here, we conducted two experiments in which eggs were subjected to: (i) natural, diurnal temperature cycles based on the results of Potter, Davidowitz & Woods (2009) or (ii) short-term, ecologically relevant heat shocks. We examined how egg temperatures affected hatching time, hatching mass, larval growth, development time, pupal mass, head capsule size and adult fecundity.

Fig. 1. A *Manduca sexta* egg on a *Datura wrightii* leaf.

Materials and methods

In the southwestern USA, *M. sexta* L. (Lepidoptera: Sphingidae) is active from July to September, during the monsoon season (Riffell *et al*. 2008). Females attach eggs (1–2 mm in diameter) singly to the lower surface of host leaves; eggs hatch after ~4 days, and larvae typically develop through 5, or sometimes 6, instars (Kingsolver 2007). In 2008 and 2009, we collected wild *M. sexta* eggs and larvae from its primary host plant, *Datura wrightii* R. (Solanaceae), around Tucson, Arizona, USA. We reared larvae for the remainder of their larval stages in outdoor (ambient temperature) boxes on cut leaves of *D. wrightii*. When larvae began their stereotyped wandering behaviour, indicating readiness to pupate, we transferred them to individual cups of potting soil for pupation. Approximately 1 week before emergence, pupae were moved from the soil into a growth chamber on a 16L : 8D cycle, cycling between 30 and 20 °C. Moths emerged in a $2 \times 2 \times 2$ m flight cage and had access to potted *D. wrightii* for oviposition and to sugar water and *Datura* nectar for food. Eggs laid by these wild moths were used for all experiments. We collected and reared over 300 wild moths each year, and there were up to 48 moths in the flight cage at a time.

EXPERIMENT 1: NATURAL TEMPERATURE CYCLES

Multiple moths were allowed to oviposit for 3 h (20:30–23:30). Eggs were collected and placed randomly into four rows of an aluminium thermal gradient bar (for bar details, see Woods & Bonnecaze 2006). Temperature gradients in the bar were set by circulating controlled-temperature water through chambers in each end. Bath temperatures were set to mimic the daily temperature cycles of *D. wrightii* leaves in Tucson, AZ (Potter, Davidowitz & Woods 2009). Gradient bar conditions were set as close as possible to those used by Potter, Davidowitz & Woods (2009), although only four of the twelve rows were used for this experiment. During the night, all rows dropped to ~25 °C. During the day, temperatures were ramped up slowly such that the rows reached peak temperatures of 31, 34, 37 or 40 °C for 3 h and then ramped back down (Fig. 2). Copper–constantan thermocouples

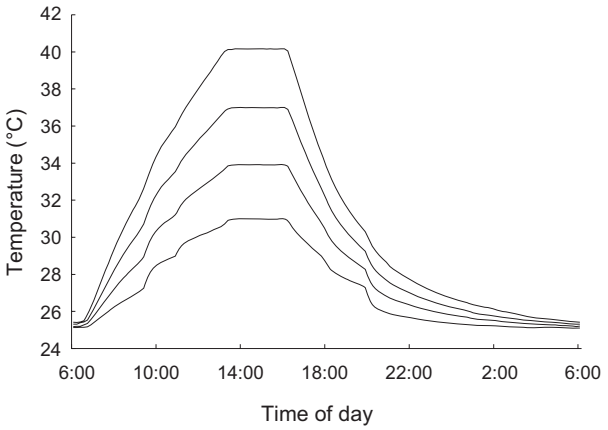

Fig. 2. Four temperature treatments for the natural temperature cycle experiment (experiment 1). Each line represents one treatment, such that eggs in the coolest treatment experienced fluctuating temperatures with a daily peak of 31 °C (bottom line), and eggs in the hottest experienced a daily peak of 40 °C (top line).

were placed in each row, and temperature was logged every 10 min. Two hours before expected hatch time (estimated from previous experiments), the eggs were removed from the bar and glued onto *D. wrightii* leaf cuttings. We used Elmer's Mucilage glue, which we found to have no effect on egg hatching. Larvae were kept in a growth chamber on a 16L : 8D cycle, cycling between 30 and 20 °C. Leaves were replaced and watered as needed. Larvae were reared individually until the final (fifth) instar, when larvae were marked with unique ID codes and moved to large bins full of *Datura* cuttings. Leaves were changed twice daily, and bins were moved around the growth chamber to minimize bin effects.

The entire experiment was repeated twice. In the first replicate (September 2008, $N = 28$), we followed individual larvae through the fourth instar. We measured 24-h growth rate during the first instar; larval mass 6, 13 and 15 days post-oviposition; and first instar head capsule width. The experiment was repeated and expanded in September 2009. One subset of eggs ($N = 15$) was placed under a webcam, to record hatch times using time-lapse video software (Flix v3·3; http://www.nimisis.com/projects/flix.php). A second subset ($N = 13$) was frozen immediately after hatching, dried at 55 °C for 24 h, and weighed to determine hatchling dry mass. The remaining eggs ($N = 35$) were glued on plants and reared to adulthood. We measured head capsule width for all instars, larval mass at 10, 17 and 18 days post-oviposition, wandering day, pupal mass, and female mass and egg load. Methods for specific measurements are described below. Results from 2008 and 2009 were qualitatively similar; here we give statistics for 2009 data except for measurements that were only collected in 2008.

EXPERIMENT 2: HEAT SHOCK

Eggs were collected after 1 h of oviposition and kept at room temperature (~23 °C). Three days later, eggs were separated into two groups and given a 2-h heat shock of 37 or 43 °C. Wild eggs under *Datura* leaves may reach 45 °C, and 100% of eggs survive a 2-h exposure to 43 °C (Potter, Davidowitz & Woods 2009). After 2 h, the eggs were returned to room temperature for the rest of development. Approximately 4 h before hatching, the eggs were glued with Elmer's Mucilage glue onto *D. wrightii* leaves. Larvae and leaves were kept in a growth chamber on a 16L : 8D cycle, cycling between 30 and 20 °C. In 2008, we measured growth rate during the first instar, and larval mass 8 days post-oviposition ($N = 14$–20). The experiment was repeated and expanded in September 2009. One subset of eggs ($N = 30$) was placed under a webcam, to record hatch times using time-lapse video software. A second subset of eggs was checked every 15 min, and larvae were weighed upon hatching ($N = 27$). The remaining eggs were glued onto plants and reared to adulthood; we measured head capsule width for all instars, first instar growth rate, larval mass 6 and 14 days post-oviposition, wandering day, pupal mass, and mature egg load for females ($N = 16$). Methods for specific measurements are described below. Results from 2008 and 2009 were qualitatively similar; here we give statistics for 2009 data except for measurements that were only collected in 2008.

GROWTH RATE

We measured larval growth during the middle 24 h – the linear growth phase – of their first instar (Nijhout, Davidowitz & Roff 2006). Eggs hatched over ~20 h, and the first instar lasts only ~2 days. Hatching larvae were checked every 12 h to catch the linear phase. In experiment 1, we measured each larva's initial weight 12 h after its first feeding (typified by obvious green in its gut), and again 24 h later. In experiment 2, we measured each larva's weight 12 h after all larvae had started feeding, and then again 4 h later. In addition, we measured 24 h growth during the linear growth phase of the fifth instar in experiment 1. For each measurement period, growth rate [$(\text{mass}_2 - \text{mass}_1)/\text{hours}$] was compared for larvae from different egg treatments.

HEAD CAPSULES

When possible, we collected newly shed head capsules from larvae immediately after they moulted (Fig. 1). Head capsules were photographed from above through a stereomicroscope (Nikon SMZ1500 with DS-5M camera, Nikon Corporation, Tokyo, Japan). At each magnification used, we also photographed a stage micrometre so that other photos from the same magnification could be calibrated. Head capsule width was measured at the widest part of the head using ImageJ (NIH software, Bethesda, MD, USA). All capsules were oriented the same way, and measurements and analyses were performed blind. Because some capsules were lost or damaged before collection, sample sizes varied across instar. We analysed head capsule data with linear mixed effect (LME) models (Pinheiro & Bates 2000) implemented in the R statistical package (R Development Core Team 2010). LME analyses provide an appropriate and flexible way to deal with repeated observations on individuals, especially because, for some individuals, some head capsule data are missing.

WANDERING DAY AND PUPAL WEIGHT

When they are ready to pupate, *M. sexta* larvae begin a stereotyped wandering behaviour. Larvae were checked daily and upon wandering were moved to individual 9-oz cups with damp potting soil. Pupae were held at ~23 °C and were weighed and sexed on day 10 post-wandering.

FEMALE POTENTIAL FECUNDITY

Female pupae from experiments 1 and 2 were reared to eclosion. Twenty-six days after wandering, pupae were transferred to individual paper bags and kept on a 16L : 8D cycle. Bags were checked every 12 h, and moths were weighed upon eclosion. Moths were returned to

their bags for 72 h, weighed again and then frozen. Preliminary experiments on *M. sexta* from a laboratory colony showed that egg number reaches a maximum ~72 h post-eclosion (G. Davidowitz, unpublished); females subsequently begin to resorb them. We dissected their abdomens and counted mature (chorionated) eggs. For experiment 1, moths were kept at 25 °C and ~20% RH. For experiment 2, moths were kept at 25 °C and ~60% RH.

Results

EXPERIMENT 1: NATURAL TEMPERATURE CYCLES

Although each treatment was given fluctuating temperatures throughout egg development, for simplicity, we refer to each treatment by its peak daily temperature, which was held for 3 h each day (Fig. 2; four treatments). Consistent with Potter, Davidowitz & Woods (2009), temperature had a strong effect on egg development time. Eggs at 34 and 37 °C had the shortest time to hatching (average ~88 h), and eggs at warmer and cooler temperatures had longer development times (up to 105 h). The best-fit equation for egg development time was a second-order polynomial (Fig. 3a; $N = 15$ each; $R^2 = 0{\cdot}64$).

Hatchlings from eggs exposed to higher temperatures had smaller head capsules (Fig. 3b; $N = 15$–24 each; $P < 0{\cdot}0001$; $R^2 = 0{\cdot}55$) and lower dry mass (Fig. 3c; $N = 10$–13 each; $P = 0{\cdot}015$; $R^2 = 0{\cdot}12$). An LME analysis of all head capsule data indicated that the effect of temperature on head capsule size faded as development progressed (Fig. 4a, significant temperature × instar effect in Table 1a). *Post hoc* ANOVA tests suggested that the head capsule size difference persisted until the fourth instar ($P < 0{\cdot}001$ for instars 1–3).

Egg temperature affected growth rate of larvae during their first instar (Fig. 3d; $N = 22$–23 each; $P = 0{\cdot}0002$; $R^2 = 0{\cdot}18$); smaller hatchlings from eggs exposed to higher temperatures grew the slowest. By the fifth instar, growth rates were equivalent (Fig. 3g; $P = 0{\cdot}43$), however, mirroring the diminishing effect of egg temperature on head capsule size over time. Because of the variation in hatch times and initial growth rates, a larva's mass at any number of days post-oviposition was strongly determined by its egg temperature. The best-fit equation for all larval mass measurements (i.e. on days 6, 10, 13, 15, 17 and 18) was a second-order polynomial (always $P < 0{\cdot}001$); larvae from 40 °C eggs were always the smallest [see mass in the third instar (measured on day 10) shown in Fig. 3e; $P < 0{\cdot}0001$; $R^2 = 0{\cdot}20$]. This was reflected in the total days from oviposition until wandering (beginning of pupation). Larvae from warmer eggs wandered later than those in cooler treatments (Fig. 3f; $N = 28$–33 each; $P = 0{\cdot}01$; $R^2 = 0{\cdot}07$). There was no difference in final pupal mass (Fig. 3h; $N = 28$–33 each; $P = 0{\cdot}43$) or female poten-

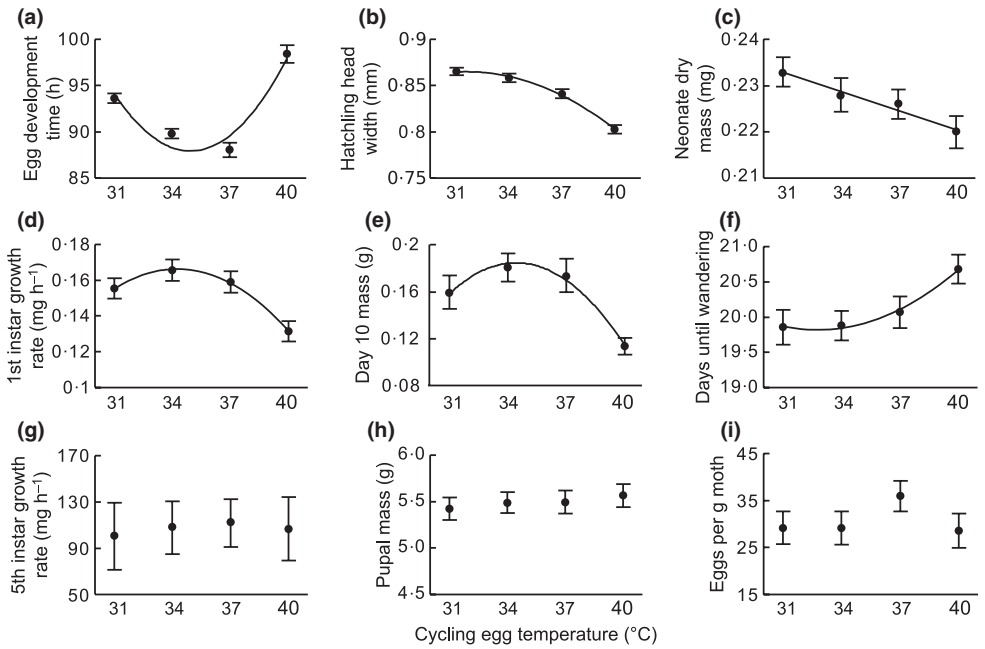

Fig. 3. Results for experiment 1. *Manduca sexta* eggs were exposed to temperature cycles mimicking those experienced on host plants (31, 34, 37 or 40 °C daily maximum egg temperature), and larvae were reared on *Datura wrightii* in a common garden after hatching. All points are mean ± SE. (a) Development time for eggs in each of the four temperature treatments ($N = 15$ each). The curved line shows the best-fit polynomial for egg development time ($R^2 = 0{\cdot}64$; $y = 0{\cdot}39x^2 - 27{\cdot}6x + 570$). (b) Dry mass of hatchlings ($N = 10$–13 each). Larvae were frozen upon hatching and dried at 55 °C for 24 h ($P = 0{\cdot}015$; $R^2 = 0{\cdot}12$; $y = -0{\cdot}0013x + 0{\cdot}27$). (c) Head capsule width for first instar larvae ($N = 15$–24 each; $P < 0{\cdot}0001$; $R^2 = 0{\cdot}61$; $y = -0{\cdot}0009x^2 + 0{\cdot}056x - 0{\cdot}02$). (d) Larval growth rate during the linear growth phase of the first instar ($N = 22$–23 each; $P = 0{\cdot}0002$; $R^2 = 0{\cdot}18$; $y = -0{\cdot}025x^2 + 1{\cdot}72x - 25{\cdot}5$). (e) Larval mass during the third instar, 10 days after oviposition ($N = 18$–24 each; $P < 0{\cdot}0001$; $R^2 = 0{\cdot}20$; $y = -0{\cdot}0022x^2 + 0{\cdot}15x - 2{\cdot}5$). (f) Mean days from oviposition until wandering (beginning of pupation) ± SE ($N = 28$–33 each). (g) Larval growth rate during the linear growth phase of the fifth instar ($P = 0{\cdot}43$). (h) Mass of pupae on day 10 post-wandering ($N = 28$–33 each; $P = 0{\cdot}43$). (i) Female potential fecundity (mature eggs per g moth), measured 72 h post-eclosion ($N = 10$–13 each; $P = 0{\cdot}71$).

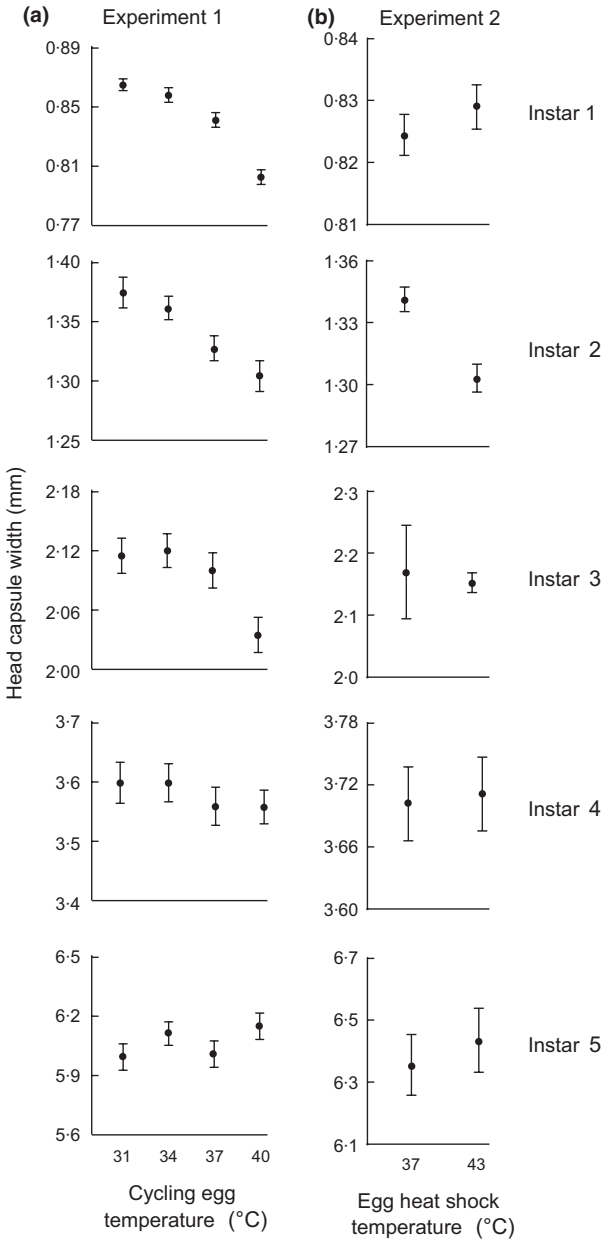

Fig. 4. (a) Head capsule width for *Manduca sexta* instars. Larvae were given four cyclical temperature treatments during the egg stage (31, 34, 37 or 40 °C maximum daily temperature), and were reared on *Datura wrightii* in a common garden after hatching. Head capsule sizes differed at hatching, and this difference persisted until the fourth instar (*post hoc* ANOVA tests; $P < 0{\cdot}001$). (b) Head capsule widths for larvae from eggs that received a 2-h heat shock of 37 or 43 °C on day 3 of development. Eggs and larvae were reared in a common garden except for the 2-h egg heat shock. Capsule size diverges at the second instar (*post hoc t*-test; $P < 0{\cdot}001$). All points are mean ± SE.

tial fecundity (eggs per g moth; Fig. 3i; $N = 10$–13 each; $P = 0{\cdot}71$).

EXPERIMENT 2: HEAT SHOCK

Here, *M. sexta* were raised in a common garden for their entire development, except for 2 h during the egg stage. Eggs received a 2-h heat shock of either 37 or 43 °C. This short temperature difference was enough to delay hatching in the warmer group by 4·3 h (Fig. 5a; 37 °C mean: 92·7 ± 0·59 h; 43 °C mean: 97·0 ± 0·50 h; $N = 30$ each; $P < 0{\cdot}0001$). While egg temperature did not affect hatchling mass (Fig. 5b; $N = 27$ each; $P = 0{\cdot}53$), the two groups differed in their growth rate (Fig. 5c; $N = 16$; $P = 0{\cdot}0024$). The slower growth rate of the 43 °C group magnified their ~4 h delay in hatching, so that larvae from the warmer eggs were smaller at every mass measurement (e.g. Fig. 5d,e; $N = 16$; $P < 0{\cdot}01$) and they wandered 1·2 days later than the 37 °C group (Fig. 5f; $N = 16$ each; $P < 0{\cdot}01$). Pupal mass and potential fecundity did not differ between treatments (Fig. 3g,h; $P = 0{\cdot}51$ and 0·88 respectively). In contrast to experiment 1, a 2-h egg heat shock did not affect initial head capsule size (Fig. 4b). The data suggested little temperature-driven difference subsequently, and an LME analysis of the full data set confirmed this impression (Table 1b). Nonetheless, when we performed another LME on just the first two instars, a strong temperature × instar interaction emerged (Table 1c). The two LMEs were inconsistent probably because the effect of temperature on the second instars was small.

Table 1. (a) Summary of linear mixed effect (LME) model for experiment 1. See text for methods; the number of collected head capsules varied across instars ($N = 27$, 39, 70, 51 and 116 for instars 1–5, respectively; a total of 116 larvae were in the experiment). The effect of egg temperature on head capsule size faded as development progressed. (b, c) LME results for experiment 2 ($N = 23$, 24, 30, 32 and 25 collected capsules for instars 1–5, respectively; a total of 32 larvae in the experiment). Although LME analysis of all instars (b) showed little effect of egg temperature, LME analysis on just the first two instars (c) showed a strong temperature × instar interaction. In all LME models, temperature was treated as a covariate and instar as a factor

Variable	Numerator d.f.	Denominator d.f.	*F*	*P*
(a) Cycling egg temperature				
Intercept	1	179	137767·6	< 0·0001
Temperature	1	114	410·3	< 0·0001
Instar	4	179	21133	< 0·0001
Temperature × instar	4	179	7·2	< 0·0001
(b) Egg heat shock (all instars)				
Intercept	1	94	59491·8	< 0·0001
Temperature	1	30	0·24	0·63
Instar	4	94	14357·2	< 0·0001
Temperature × instar	4	94	1·4	0·23
(c) Egg heat shock (instars 1 and 2)				
Intercept	1	23	294·3	< 0·0001
Temperature	1	23	0·054	0·82
Instar	1	20	17170·7	< 0·0001
Temperature × instar	1	20	25·7	0·0001

Discussion

In insects, temperature-driven plasticity is ubiquitous (Ananthakrishnan & Whitman 2005; Whitman & Ananthakrishnan 2009), and cross-stage effects are well known. For example, many studies now demonstrate that exposure to different

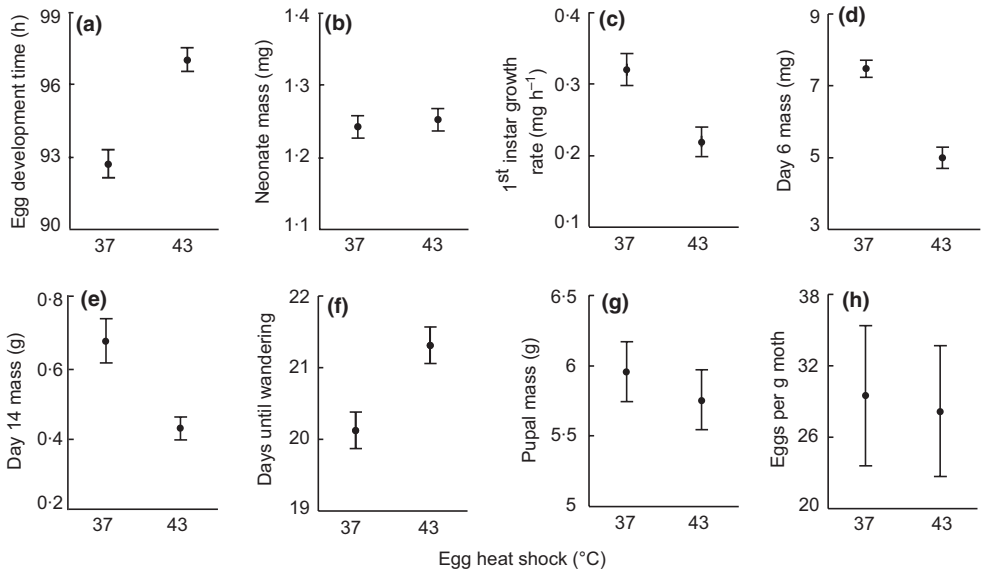

Fig. 5. Data for *Manduca sexta* reared on *Datura wrightii* in a common garden except for a single 2-h heat shock of 37 or 43 °C during the egg stage. All points are mean ± SE. (a) Development time for eggs (oviposition until hatch). The hotter heat shock delayed hatching by 4·3 h (37 °C mean: 92·7 ± 0·59 h; 43 °C mean: 97·0 ± 0·50 h; $N = 30$ each; $P < 0{\cdot}0001$). (b) Mass of *M. sexta* hatchlings ($N = 27$ each; $P = 0{\cdot}53$). (c) Larval growth rate during the linear growth phase of the first instar ($N = 16$ each; $P = 0{\cdot}0024$). (d) Larval mass during the first instar, 6 days (136 h) after oviposition. $N = 16$ each; $P < 0{\cdot}0001$. (e) Larval mass during the fourth instar, 14 days (327 h) after oviposition. $N = 16$ each; $P < 0{\cdot}01$. (f) Days from oviposition until wandering (beginning of pupation). The hotter heat shock delayed wandering by an average of 1·2 days (37 °C mean: 20·1 ± 0·25 days; 43 °C mean: 21·3 ± 0·25 days; $N = 16$ each; $P < 0{\cdot}01$). (g) Mass of pupae on day 10 post-wandering ($N = 16$ each; $P = 0{\cdot}51$). (h) Female egg load (mature eggs per g moth) 72 h post-eclosion ($N = 7$–8 each; $P = 0{\cdot}88$).

temperatures during the larval stage can influence the adult phenotype (Crill, Huey & Gilchrist 1996; Imasheva *et al.* 1997; Roberts & Feder 1999; Birkemoe & Leinaas 2000; Davidowitz, D'Amico & Nijhout 2004). Our study goes back further in development to ask whether experience in the earliest life-history stage – the egg stage – induces plasticity and, if so, how far into the rest of the life cycle that plasticity persists.

We subjected eggs of *M. sexta* either to natural, diurnal temperature cycles (Fig. 2), or to ecologically relevant heat shocks, and then reared them in a common garden after hatching. In both experiments, slight differences in egg temperature affected development time and therefore presumably fitness (as discussed below). Egg temperature had strong effects on embryos and early larval instars. However, temperature's effects rapidly faded in later instars and, except for differences in the timing of pupation, were entirely absent from pupal and adult stages. Compensatory growth and uncoupled life stages may help *Manduca* minimize the long-term phenotypic consequences of early thermal stress.

CYCLING TEMPERATURES VS. HEAT SHOCK

Both temperature manipulations – cycling and heat shock – affected embryo development time. The two experiments differed, however, in the pattern and timing of temperature's other effects. For eggs in cycling temperatures, effects were pronounced in hatchlings (mass, head capsule size and growth rate; Fig. 3b,c,d) but had disappeared by the fourth instar. For shocked eggs, effects were minimal in hatchlings (growth rate, Fig. 5c), somewhat larger in the second instar (head capsule size, and presumably mass, see below; Fig. 4b), and gone in subsequent instars. Both patterns can be explained by the timing of larval moults. In *Manduca*, head capsules do not grow within an instar, and the mass of a larva when it moults determines the size of its next head capsule (Nijhout 1975, 1981). In the cycling temperature experiment, larvae from hotter eggs likely reached a higher mass relative to their head width before committing to moult. Because they also grew more slowly, they presumably grew for longer periods before committing to some or all of their first three moults. If smaller hatchlings delayed moulting (the endocrine events underlying moulting depend on photoperiod gates; Truman 1972), larvae from the four treatments could converge in head capsule size. In contrast, a short heat shock late in the egg stage did not affect the already-formed first instar head or body in the same way as cycling temperatures did. Rather, a 43 °C shock probably resulted in stressed hatchlings that grew slower, moulted at smaller sizes and subsequently formed smaller head capsules during the first-to-second instar moult.

Among ectotherms, low developmental temperature often results in slower growth, longer development time and larger adult size. The latter relationship – cold development causing large adult size – is known as the temperature-size rule (TSR) (Atkinson 1994; Sibly & Atkinson 1994; Blanckenhorn 2000; Angilletta & Dunham 2003). Recent studies, including ours, suggest that the mechanisms underlying the TSR apply to the egg stage as well: hatchlings are larger in cooler temperatures (e.g. Geister *et al.* 2009). This consistent pattern of phenotypic plasticity may be a result of adaptation to different ther-

mal environments (Fischer, Brakefield & Zwaan 2003), or an unavoidable consequence of a temperature effect during development (reviewed in Chown & Gaston 2010). While the underlying mechanisms or the potential adaptive significance are still unresolved (Fox & Czesak 2000; Steigenga & Fischer 2007), a possible explanation is the biophysical model by Van Der Have & De Jong (1996) (see also Ernsting & Isaaks 1997, 2000; Walters & Hassall 2006). According to their model, cells divide more rapidly at higher temperatures, while cellular growth increases less rapidly. An intriguing question, therefore, is whether *M. sexta* hatchlings from warmer eggs are small because they have smaller cells.

CROSS-STAGE CONSEQUENCES

Long-term effects of embryonic temperature are well known and frequently observed in birds, reptiles, mammals and fish. Why were they so modest here? One possibility is that insects, more than other studied organisms, are better able to tolerate environmental insults during the egg stage. Unlike many vertebrates, most insect embryos receive little to no protection from the environment via parental care. The temperature of insect oviposition sites can vary tremendously, both within the life of an individual egg (Roberts & Feder 1999; Gibbs, Perkins & Markow 2003; Potter, Davidowitz & Woods 2009) and between generations (Roy & Thomas 2003). In addition, insect eggs are small and, for terrestrial insects, often deposited in exposed habitats; both attributes maximize the potential for rapid changes in temperature. The hypothesis that insect eggs are more tolerant of environmental insults predicts a correlation between degree of parental care and offspring growth physiology, and, in fact, some data do indicate this. For example, eggs that do not rely on parental protection have accelerated cell division (Hamdoun & Epel 2007), allowing them to more quickly reach a stage at which they can respond to their environment.

After hatching, modular life cycles may allow insects to mitigate the consequences of temperature by uncoupling its physiological effects from one life stage to the next; we call this the *lifecycle modularity hypothesis*. A key difference between insects and most other taxa studied is that insects' life stages are punctuated by periods of time (moults) in which the insect ceases other activities and focuses, physiologically speaking, on expanding and rebuilding. Compared to the incremental development of most vertebrates, insects' modular development may allow more radical isolation of disturbance in one stage from physiology in the next. For example, while a vertebrate's skeleton is built on a foundation that is formed during embryogenesis, an insect's exoskeleton is reconstructed at every moult. The *lifecycle modularity hypothesis* is also suggested for the lack of relationship between early stressors and adult fluctuating asymmetry, a commonly used indicator of stress. Particularly in organisms with complex life cycles, an early larval stress often does not predict fluctuating asymmetry in the adult stage (Floate & Fox 2000; Servia, Cobo & González 2002; Piscart, Moreteau & Beisel 2005; Dongen 2006; Campero *et al.* 2008). Tests of the modularity hypothesis could compare how metamorphic vs. non-metamorphic – or progenetic vs. non-progenetic – sister taxa respond to stress, focusing particularly on whether metamorphosis insulates later stages. A simpler comparative experiment could examine whether, in insects, adults of species with fewer instars are less buffered than those with many instars.

For animals with modular development, rapid compensation for poor early conditions may be particularly beneficial. An insect's survival and fecundity depends strongly on its body size at a precise point – the transition from larva to adult (Taylor, Anderson & Peckarsky 1998). For organisms that grow incrementally, that limitation does not exist. Here, small *Manduca* hatchlings fully compensated for the effects of suboptimal egg conditions by lengthening their first few instars. The benefits of catching up – particularly by the fifth instar – are large: a larva's mass at the moult to fifth instar determines its maximum potential pupal mass (Nijhout, Davidowitz & Roff 2006), which in turn is strongly correlated with potential fecundity (Davidowitz, unpublished; Diamond & Kingsolver 2010). Similar compensatory growth has also been observed in other metamorphosing taxa: temperature-related delayed hatching in frogs triggers faster growth in larvae and juveniles, resulting in nearly total compensation for embryonic temperature effects (Orizaola, Dahl & Laurila 2010). In frogs, fitness increases with both earlier metamorphosis and larger size at metamorphosis (Altwegg & Reyer 2003), and indeed, they compensate in this way.

While compensatory growth has clear benefits, it is often associated with cellular or ecological costs (Metcalfe & Monaghan 2001). Here, compensation occurred by lengthening, not speeding, growth; indeed, smaller larvae grew more *slowly*. Cellular costs are therefore unlikely, and compensation had no impact on egg production, a direct measure of potential fecundity. The obvious cost is increased time of exposure to predators and parasitoids (Bernays 1997; Mira & Bernays 2002). In nature, these smaller hatchlings may be able to catch up more quickly by seeking out warmer leaves. While larvae in our study were reared in a common garden, larvae in nature can thermoregulate by moving to different microclimates within a plant (Casey 1976; Capinera, Wiener & Anamosa 1980; Kührt, Samietz & Dorn 2005). In turtles, hatchlings from suboptimal egg environments may increase their growth rate by spending more time in warmer areas (O'Steen 1998). Regardless of any post-hatching activities, however, egg temperature still could affect fitness in nature. Eggs are highly vulnerable to predation: for *M. sexta*, typically 20–45% (but up to 70% in some areas) of mortality is egg related (Mira & Bernays 2002). Therefore, prolonged egg development, even by part of a day, may increase risk.

In conclusion, our experiments using *M. sexta* demonstrate that ecologically relevant egg temperatures induce phenotypic plasticity in multiple, performance-related traits. The effects are strong on embryos and early larval instars, but disappear rapidly in the absence of temperature differences during subsequent life stages. Compensatory growth and uncoupled life stages may help insects cope with environmental variability

and reduce the effects of early thermal stress in subsequent stages.

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