Egg parasitoids face unique developmental constraints. First, they have exceptionally limited resources to support themselves and their siblings through three life stages. Second, they develop within the physiological system of another species, which they modify to their own ends. We examined how these constraints affect the metabolic physiology of egg parasitism, and whether parasitoids retool their host eggshell to account for their different metabolic demands. Higher-conductance eggshells allow more oxygen to reach the developing parasitoids, but also allow more water to leave the egg. We used Manduca sexta (Lepidoptera: Sphingidae) eggs and Trichogramma (Hymenoptera: Trichogrammatidae) parasitoids from southeastern AZ, USA. Compared with unparasitized Manduca eggs, eggs parasitized by Trichogramma had lower peak metabolic rates and approximately 50 per cent lower metabolic efficiency. However, developing Trichogramma were far more efficient than typical transfer efficiencies between trophic levels (approx. 10%). Even within a few hours of parasitization, eggs containing more Trichogramma had lower per-parasitoid metabolic rates, suggesting that parasitoid larvae have mechanisms for rapidly adjusting their metabolic rates based on number of siblings. Parasitoids also appear to control the conductance of their host eggshell: their different metabolic demands were mirrored by shifts in rates of water loss.

Keywords: efficiency; idiobiont; Manduca sexta; metabolism; trophic transfer; water balance

1. INTRODUCTION

Compared with many animals, parasitoids face two unusual developmental constraints. First, they kill, consume and develop within only one individual host, a uniquely restricted resource. Second, they live in physiological systems evolved to support other insects (their hosts), which they modify to their own ends. How parasitoids hijack the behaviour, growth and metabolism of larval and adult hosts is relatively well studied [1,2]. How parasitoids of eggs retool their hosts’ physiology to their own ends is unknown. To our knowledge, this is the first study on the metabolic physiology of egg parasitism.

The metabolic physiology of egg parasitoids places important constraints on their life-history traits. In an unparasitized egg, nutrients and water support the development of a single individual; in a parasitized egg, by contrast, those resources often are partitioned among multiple developing parasitoids. Also, in an unparasitized egg, the resources support a single life stage—the embryo; in a parasitized egg, the resources must support each individual parasitoid for longer—through the embryo, larva and pupa. Although parasitoids consume all egg resources by the end of the larval stage, their pupae must rely on resources entirely derived from the host egg (and stored in the larvae) for metabolism and metamorphosis.

Here, we test a set of hypotheses about how the life-styles of egg parasitoids are related to two core aspects of their metabolic physiology: efficiency and peak power. Metabolic efficiency is defined as the fraction of non-water egg resources that ends up in the hosts or parasitoids that emerge. Peak power is the maximum metabolic rate during development. A reasonable hypothesis is that constraints on parasitoid lifestyles—i.e. prolonged development of more individuals on finite resources—have led to the evolution of high efficiency and low peak power. For example, parasitoids could absorb and assimilate food with higher efficiency, which would allow them to use more of the available resources and increase their potential mass at hatching. Alternatively, immature parasitoids may have low efficiency and high peak power, as smaller individuals, in general, have higher mass-specific metabolic rates [3]. Collectively, therefore, many small parasitoids, each with higher mass-specific metabolism, may show higher absolute metabolic rate (per egg) compared with unparasitized hosts. Competition with siblings, even if they are related, may also cause individual parasitoids to trade off efficiency for power [4]. A third possibility is that peak power and overall efficiency do not trade off (unlike instantaneous power and efficiency [5]). If parasitoids have low peak power, they could still have lower efficiency altogether because they stay in the egg for much longer.

A complementary problem is whether and how parasitoids retool their host egg to account for their different metabolic demands. For example, if parasitoids show different timing of peak power compared with their hosts, parasitoids may also, in parallel, shift the timing of changes in eggshell conductance. Higher-conductance eggshells allow more oxygen to reach the developing parasitoids, but also allow water vapour to pass more rapidly out of the egg [6]. We test whether parasitoids’ shifts in peak power are mirrored by changes in eggshell conductance.
We distinguish these possibilities by measuring the developmental and metabolic trajectories of *Trichogramma* (Hymenoptera: Trichogrammatidae) wasps in eggs of *Manduca sexta* (Lepidoptera: Sphingidae). *Trichogramma* wasps are tiny (less than 1 mm long), yet because of their importance in biological control, their behaviour, morphology and nutritional ecology are well known [7]. We use *M. sexta* eggs from southeastern Arizona, and wild *Trichogramma* parasitoids that co-occur with this population. Our two focal species, *Trichogramma deion* and *Trichogramma sathon*, are gregarious: multiple parasitoids develop within each host egg. *Manduca* embryos hatch in approximately 4 days, emerging as larvae, while *Trichogramma* wasps remain in the eggs for twice as long, emerging as adults.

2. MATERIAL AND METHODS

(a) Animals

(i) Manduca sexta

In the southwestern USA, *M. sexta* Linnaeus (Lepidoptera: Sphingidae) is active from July to September. Females attach eggs singly to the lower surface of host leaves; eggs hatch after approximately 4 days [8]. In August 2010, we collected wild *M. sexta* larvae from their primary host plant, *Datura wrightii* Regel (Solanaceae), in the Chihuahuan Desert around Portal, AZ, USA. We reared larvae for the remainder of their larval stages on cuttings of *D. wrightii*, on a short-day light cycle (12 L : 12 D; 20°C). Larvae pupated in individual cups of soil and were in diapause (approx. 20°C) over the winter. In July 2011, we moistened the soil to break diapause; moths emerged in a 2 × 2 × 2 m outdoor flight cage at the Southwestern Research Station, near Portal and had access to cuttings of *D. wrightii* for oviposition and to diluted honey and *Datura* nectar for food. Eggs laid by these moths were used for all experiments.

(ii) Trichogramma sp.

In 2010 and 2011, we reared seven species of egg parasitoids from parasitized *Manduca* eggs collected approximately within a 20 km radius at our field site: one scelionid, four scelionids, one eupelmid, *T. sathon* and *T. deion*. The two *Trichogramma* species were the most common; although they are easily distinguished from the others, they can be distinguished from each other only by genotype [9]. In July 2011, we collected *Trichogramma* wasps from 30 parasitized *M. sexta* eggs on *D. wrightii* plants around Portal, AZ, USA. Multiple (approx. 5–40) wasps emerge from each egg; these groups were kept in separate 50 ml tubes. Twelve tubes of wasps were fed diluted honey daily and used to parasitize the eggs for all experiments. Remaining wasps were frozen after emergence; these adults were counted to confirm that laboratory and naturally parasitized eggs contained similar numbers of wasps (see the electronic supplementary material). We confirmed the identity of all wasps via genotype after the experiments, following Stouthamer et al. [9].

Briefly, we amplified the internally transcribed spacer 2 of the nuclear ribosomal gene complex and digested the resulting DNA product with Mse1 restriction enzyme. *Trichogramma sathon* and *T. deion* were differentiated based on the sizes of the PCR product and the restriction length polymorphisms, using standard agarose electrophoresis.

(b) Treatments

Moths were allowed to lay eggs overnight (19.30–5.30). Eggs were collected in the morning (hereafter ‘day 1’) and split into two groups. The first was non-parasitized. The second was exposed to *Trichogramma* parasitoids for 2 h, during which the ratio of wasps:eggs was at least 2:1 in each tube. Both groups of eggs were then kept in a rearing incubator (30°C, approx. 50% relative humidity (RH), 14 L: 10 D), except when measured in each experiment.

(c) Egg water loss

Each day (11.00), we placed a new set of eggs from both treatments in a dry chamber (30°C, approx. 1% RH). After 2 h, eggs were weighed on a microbalance (± 1 μg) and returned to the dry chamber; this initial drying eliminated water vapour adhering to the eggshell. Four hours later, eggs were removed from the dry chamber, weighed again and returned to their rearing incubator (30°C, approx. 50% RH) for the remainder of development; this 4 h mass loss rate represented water loss through the eggshell. We later scored eggs for hatching success (*Manduca* larvae hatched from non-parasitized eggs on day 4, and *Trichogramma* adults hatched from parasitized eggs on day 8). Unhatched or unsuccessfully parasitized eggs were removed from the analysis, leaving n = 10–25 per day per treatment. Each egg was measured only once.

(d) Egg respiration

CO₂ emission from individual eggs was measured using flow-through respirometry. Ten parasitized and 15 non-parasitized eggs were measured every afternoon until hatching. An additional eight parasitized eggs were measured on days 4–7. Eggs were weighed and placed singly into a water-jacketed stainless-steel chamber [10], whose temperature was maintained at 30°C. Rates of CO₂ emission were measured using a two-cell infrared analyser (Liric LI-7000, Lincoln, NE, USA) in differential mode. Dry, CO₂-free compressed air (9.5 ml min⁻¹ standard temperature and pressure, dry) was directed through the LI-7000 reference cell, then through the steel-jacketed chamber and then through the LI-7000 measurement cell. Flow rates of gas were set by a mass-flow controller (Sierra Instruments, Monterey, CA, USA 0–10 sccm, calibrated for air), connected to a set of controlling electronics (MFC-4, Sable Systems International, Las Vegas). All gases were handled using one-quarter inch outer diameter copper tubing. The analyser was calibrated regularly with pure N₂ and 100 ppm CO₂ in N₂ (Airgas). Data were logged using ExpsData software (v. 1.0.17; Sable Systems) receiving digital signals from an analogue-to-digital converter (UI2, Sable Systems), which itself received analogue signals from the instrument. We also logged temperature in a separate respirometry chamber otherwise identical to the experimental chamber except that it was fitted with a T-type thermocouple (connected to a TC-1000 meter, Sable Systems) that extended into the chamber’s air space. Each egg was measured for approximately 5 min, until the CO₂ signal was stable, and we randomized the order of eggs to control for any diurnal cycle in the embryos. CO₂ emission per egg was calculated from concentration and flow rate.

(e) Hatching dry mass

*Manduca* larvae and *Trichogramma* adults from an additional group of eggs (weighed on day 1) were frozen immediately after hatching. We counted the number of parasitoids that emerged from each egg, and then dried all hatchlings at 55°C for 48 h. Previous tests showed no further water loss after 48 h. Individuals were weighed within 1 min of removal.
from the drying oven. We compared the relationship between initial egg mass and hatchling dry mass between species.

3. RESULTS

(a) Trichogramma identification
Wasps derived from 30 field-collected eggs were used to parasitize the eggs for all experiments. Twenty-one (70%) of the eggs were identified as *T. deion* and nine were *T. sathorn*. There were no differences in any of our results by species. Data are therefore pooled and presented as *Trichogramma* sp.

(b) Egg water loss
We compared water-loss rates between species for days 1–3; the best-fit model was a second-order polynomial using log-transformed rates to account for different daily variances. Unparasitized *Manduca* eggs lost water more rapidly than eggs parasitized by *Trichogramma* (figure 1a and table 1a).

(c) Egg respiration
A linear mixed-effects analysis of log-transformed metabolic rates confirmed that, relative to eggs with intact *Manduca* embryos, eggs containing *Trichogramma* larvae had lower metabolic rates each day and also a lower peak power (figure 1b and table 2; see figure S1 in the electronic supplementary material for raw trace). On day 4, *Trichogramma* larvae pupated (see the electronic supplementary material) and showed a corresponding drop in CO₂ emission. We calculated per-parasitoid and whole-egg residuals from the daily mean metabolic rate together with the total number of wasps per egg. In eggs containing more (smaller) parasitoids, per-wasp metabolic rates were lower (figure 1c and table 1b). This difference was already significant on day 1 (table 1c). However, eggs containing more parasitoids had higher CO₂ output overall (figure 1d and table 1d).

To estimate the total metabolic output over development for each species, we integrated the area under the CO₂ curve by multiplying the means for each day by 1440 (min per day) and summing across all days (3 days for *Manduca*, 7 days for *Trichogramma*). For *Manduca*, the total was 1166.4 nmol CO₂; for *Trichogramma*, it was 3686.4 nmol CO₂, about three times higher. Because *Manduca* did not hatch until day 4 and *Trichogramma* day 8 however, this calculation misses up to 25 per cent of *Manduca* development and 13 per cent of *Trichogramma* development. *Manduca* would have had to increase their CO₂ output to more than 1.75 nmol min⁻¹ for the last day in order to approximately equal *Trichogramma*’s total output. Previous measurements of *Manduca* embryo metabolism over time (H. A. Woods 2004, unpublished data) suggest that peak rates on day 4 are less than two-thirds that high.

(d) Hatching dry mass
Developing *Manduca* used egg resources more efficiently than did *Trichogramma*—i.e. a greater fraction of initial egg dry mass ended up in a *Manduca* larva than it did in the set of wasps emerging from an egg (figure 2a). In both species, larger eggs resulted in larger total hatching mass (table 1e). There was no relationship between number of
parasitoids per egg and initial egg mass ($F = 2.13, p = 0.17; r^2 = 0.00$), or between the number of parasitoids per egg and their total dry mass ($F = 0.05, p = 0.83; r^2 = 0.14$). Dry mass per parasitoid decreased with the number of parasitoids per egg (figure 2b and table 1f).

Table 1. ANOVA summary statistics for experiments on unparasitized *Manduca sexta* eggs or *M. sexta* eggs parasitized by *Trichogramma* wasps. (In all experiments, ‘species’ refers to *M. sexta* versus *Trichogramma*; ‘day’ refers to day of development; and ‘count’ refers to number of parasitoids per egg.)

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<th>p</th>
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*aEntered in the model as a second-order polynomial.*

Table 2. Summary of linear mixed effect model for egg metabolic rate experiment (restricted to days 1–3). (‘Species’ refers to *Manduca sexta* versus *Trichogramma* and ‘day’ refers to day of development. Relative to eggs with intact *M. sexta* embryos, eggs containing *Trichogramma* parasitoid larvae had lower metabolic rates each day and also a lower peak power.)

<table>
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*aEntered in the model as a second-order polynomial.*

4. DISCUSSION

Parasitoid lifestyles are uniquely challenging: they develop within the physiological system of another species, with limited resources to support themselves and their siblings through three life stages. We examined how these constraints...
affect the metabolic physiology of *Trichogramma.* Compared with unparasitized *Manduca* eggs, eggs parasitized by *Trichogramma* had lower peak metabolic rates and lower metabolic efficiency (figures 1b and 2a). Although parasitoids lost less dry mass as CO₂ each day, they also spent much longer inside the egg and therefore emitted more total CO₂.

Parasitoids were only approximately 50 per cent as efficient as their hosts (figure 2a). In ecological food webs, the transfer efficiency from one trophic level to the next is typically approximately 10 per cent [11]. In our study, it was nearly five times higher. In this context, therefore, *Trichogramma* have extraordinarily high efficiency. By attacking their hosts early in development, egg parasitoids effectively skip a trophic level: parasitoids consume the raw materials that would become their prey, rather than eating the prey itself.

Because the two species live in the egg for different numbers of life stages, this study compares a situation of ‘growth only’ for *Manduca* versus ‘growth plus metamorphosis’ for *Trichogramma.* However, the egg resources (energy and water) do have to support *Trichogramma*’s pupal stage, unlike the pupae of *Manduca,* which depend on resources acquired after hatching. Alternative definitions of metabolic efficiency are possible: for example, we could compare the amount of mass produced per unit time per unit starting material, or compare only the growth phases. By the former definition, it is unclear which species is more efficient. By the latter definition, *Trichogramma*’s efficiency would presumably be higher, and therefore even more like skipping a trophic level. Here, however, the definition of metabolic efficiency measures ‘mass out’ as a function of ‘mass in’, which is useful in comparing how two species with distinct lifestyles make use of the same starting resources.

The metabolic data also suggest that parasitoids within an egg express some early signal of ‘crowdedness’ and rapidly adjust to how they use resources. Throughout development, eggs containing more *Trichogramma* had lower per-parasitoid metabolic rates (figure 1c). Late in development, this pattern is unsurprising, as crowded parasitoids pupate at smaller sizes (figure 2b). Remarkably, however, this pattern was already established on day 1, when *Trichogramma* larvae were just beginning to grow. Because *Trichogramma* siblings are highly related—they are haplodiploid and the majority in each egg is female—low intra-egg competition may increase each larva’s inclusive fitness.

A secondary problem is eggshell conductance. Parasitoids take over a structure (the eggshell) otherwise evolved for, and controlled by, an intact *Manduca* embryo. Can parasitoids alter that structure to support their own metabolic trajectory? Our data suggest so: in parasitized eggs, both CO₂ emission and water loss reach peaks at the same time (day 4), and both curves have similar shapes over time (figure 1a,b). Although the host embryo is killed, the rates of water loss still rise as metabolism rises. This implies either of two interesting possibilities. The first is that *Trichogramma* have evolved sophisticated means of hijacking whatever (unknown) signals *Manduca* embryos normally use to alter the conductance of their eggshells. The second is that the controls over conductance depend on factors that would be altered by developmental changes in metabolism by any egg occupant. These factors could be low oxygen or high CO₂, both of which are natural consequences of rising metabolic rates over development.

In this study, we did not measure eggshell conductance per se, although changes in conductance do contribute to changes in water loss. Early in development, water-loss rates should follow conductance, and indeed, water-loss rates for unparasitized eggs are similar to previous direct measurements of conductance [12]. Late in development, we could not distinguish whether *Trichogramma* eggs had decreased conductance, or whether they simply had less water to lose. The low initial rates of water loss suggest that *Trichogramma* larvae decrease their eggshell conductance to conserve water. Yet, *Trichogramma* can develop successfully—even outside of their host eggs, once they pupate—in approximately 0 per cent RH (see the electronic supplementary material).

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**REFERENCES**


3 Schmidt-Nielsen, K. 1984 *Scaling: why is animal size so important?* Cambridge, UK: Cambridge University Press.


