

Provided for non-commercial research and educational use only.  
Not for reproduction or distribution or commercial use.



This article was originally published in a journal published by Elsevier, and the attached copy is provided by Elsevier for the author's benefit and for the benefit of the author's institution, for non-commercial research and educational use including without limitation use in instruction at your institution, sending it to specific colleagues that you know, and providing a copy to your institution's administrator.

All other uses, reproduction and distribution, including without limitation commercial reprints, selling or licensing copies or access, or posting on open internet sites, your personal or institution's website or repository, are prohibited. For exceptions, permission may be sought for such use through Elsevier's permissions site at:

<http://www.elsevier.com/locate/permissionusematerial>

# Insect eggs at a transition between diffusion and reaction limitation: Temperature, oxygen, and water

H. Arthur Woods<sup>a,\*</sup>, Roger T. Bonnecaze<sup>b</sup>

<sup>a</sup>Section of Integrative Biology, The University of Texas at Austin, Austin, TX 78712, USA

<sup>b</sup>Department of Chemical Engineering, The University of Texas at Austin, Austin, TX 78712, USA

Received 22 November 2005; received in revised form 30 June 2006; accepted 11 July 2006

Available online 15 July 2006

## Abstract

In diverse animal taxa, eggs and embryos are incapable of transporting oxygen by convection. In such cases, internal oxygen distributions are determined jointly by rates of oxygen consumption and diffusive transport. Here we develop a mathematical model of oxygen consumption and transport in insect eggs, with the goal of understanding—for eggs in variable-temperature environments—the interactive effects of the two processes on development. We fit the model to previously published data on development time of eggs of a sphingid moth, *Manduca sexta*. The fitted coefficients suggest that eggs develop at a transition point between reaction- and diffusion-limitation. We test then this conclusion with independent data on development times of eggs distributed across a set of temperatures generated by a thermal gradient bar. Finally, we develop an extension of the model that considers tradeoffs between oxygen transfer to eggs versus water loss from them. The model results provide both a rationale for why development is often mass-transfer limited and a set of new predictions about oxygen–water tradeoffs.

© 2006 Published by Elsevier Ltd.

**Keywords:** Temperature; Eggshell; Diffusion; *Manduca sexta*; Water loss

## 1. Introduction

In aerobic organisms, metabolism is fueled by oxygen consumption. This self-evident assertion belies a set of interesting and general questions about internal oxygen economy—namely, how do organisms balance supply and demand, and how often and under what circumstances does supply versus demand limit metabolism? Such questions motivate work on diverse phenomena, including invertebrate (Pörtner, 2001, 2002) and human physiology (Richardson, 2000; Roca et al., 1989), the composition of freshwater invertebrate communities (Chapman et al., 2004), and the design of reactors for biotechnology applications (Fassnacht and Pörtner, 1999). Moreover, supply–demand considerations have stimulated important

new theory about the scaling of metabolism with body size (West et al., 1997).

We focus here on biological systems in which diffusion and reaction, rather than convection, are known to dominate oxygen distribution. Essentially all animals go through one or more stages in which internal convection is unimportant—early in ontogeny, before organs or circulatory systems have developed (Kranenbarg et al., 2000). Moreover, oxygen diffusion and consumption are differentially sensitive to temperature (Snyder, 1908, 1911; Raven and Geider, 1988): diffusion is relatively insensitive whereas reactions, including overall metabolism, often are quite sensitive. For organisms exposed to rapid or high-magnitude temperature variation relative levels of oxygen supply and demand may vary profoundly (Pörtner, 2001, 2002). This problem is faced by any metabolizing structure dependent on diffusive oxygen supply (Woods, 1999).

We have recently begun to investigate oxygen supply to eggs of an insect, *Manduca sexta* (Lepidoptera: Sphingidae) (Woods and Hill, 2004; Woods et al., 2005), a hawkmoth occurring widely in North and South America (Ferguson

\*Corresponding author. Present address: Division of Biological Sciences, University of Montana, Missoula, MT, USA.  
Tel.: +512 471 6201; fax: +512 471 3878.

E-mail addresses: [art.woods@mso.umt.edu](mailto:art.woods@mso.umt.edu),  
[art.woods@mail.utexas.edu](mailto:art.woods@mail.utexas.edu) (H. Arthur Woods),  
[bonnecaze@che.utexas.edu](mailto:bonnecaze@che.utexas.edu) (R.T. Bonnecaze).

et al., 1999; Rothschild and Jordan, 1903). Oxygen flux into *Manduca* eggs occurs by diffusion, and the main resistance to flux appears to stem from just one or two subchoral layers in the eggshell (Woods et al., 2005). Once across the shell, oxygen is consumed by embryonic tissues and, to a lesser extent, by cellularized yolk (Lamer and Dorn, 2001). Despite their small size (~1.5 mg), eggs at realistically warm temperatures (32–37 °C) exhibited clear symptoms of oxygen limitation arising from inadequate diffusive transport—extra oxygen supplied externally stimulated metabolism (Woods and Hill, 2004). At lower temperatures (22 and 27 °C), extra oxygen had no effect, implying that metabolism was limited instead by internal rates of reaction or by the supply or removal of some other metabolite. At all temperatures, moderate hypoxia (9–15 kPa O<sub>2</sub>) extended total developmental time. These results together suggest that eggs are oxygen-transport limited under some conditions but not others.

Here, we develop a mathematical model of insect egg development, with the goal of understanding the relative importance of oxygen consumption and transport, and their interaction, to development. The model is derived from simple expressions describing mass-transfer by diffusion and consumption by saturating metabolic reactions (Michaelis–Menten kinetics). A non-dimensional form of the model is derived that contains four coefficients, which we fit to previously published data on development time of embryos of *M. sexta* as a function of ambient oxygen availability (Woods and Hill, 2004). The fitted coefficients suggest that eggs develop at a transition point between reaction- and diffusion-limitation. To test this conclusion with independent data, we measure development times of eggs distributed across a finely-incremented set of temperatures generated by a thermal gradient bar and analyse the data by means of an Arrhenius plot. Finally, an extension of the model that considers tradeoffs—between oxygen transfer to eggs versus water loss from them—provides both a rationale for why development is often mass-transfer limited and a set of testable predictions about oxygen–water tradeoffs.

## 2. Model

Consider the egg of *M. sexta* as a spherical container of radius  $a$ , surface area  $A = 4\pi a^2$  and volume  $V = 4\pi a^3/3$ . The eggshell is composed of the chorion, trabecular and wax layers, and several other layers (see Woods et al., 2005, for details). The embryo, yolk and water reside in the interior. We consider the egg as a reaction–diffusion system, where all mass-transfer resistance is localized in the shell, and reaction occurs in the interior, where the oxygen concentration is assumed to be constant. These assumptions are reasonable in light of previously published data (Woods et al., 2005) showing that most of the resistance to oxygen flux occurs across the eggshell itself. Moreover, direct measurement of radial oxygen profiles within eggs of *M. sexta* (Woods and Hill, 2004) demon-

strated that oxygen gradients were steep across the eggshell, but much flatter within the egg interior.

Reaction and diffusion will be assumed to occur at steady state (or quasi-steady state) throughout development. This assumption too is reasonable. Development of the embryo requires 3–8 days depending on temperature, whereas reaction and diffusion change on much shorter time-scales (minutes to hours). Oxygen transients could arise even over these short time-scales if metabolic rates changed much more rapidly than diffusive transport could establish new, equilibrium oxygen gradients. Such a situation, however, is unlikely, as metabolic rates increase only gradually over the course of several days (Woods et al., 2005). Moreover, unpublished measurements (B. Zrubek and H. A. Woods) indicate that experimental changes in ambient oxygen availability are detectable within seconds by oxygen microelectrodes inside an egg—implying that diffusion would rapidly drive oxygen levels to new steady-state equilibria.

The overall rate of mass-transfer of oxygen  $R_m$  from the atmosphere across the shell and into the egg is proportional to the concentration difference and is given by the expression

$$R_m = k_m A (P_{O_2} - HC_I), \quad (1)$$

where  $k_m$  is the mass-transfer coefficient,  $P_{O_2}$  is the ambient partial pressure of oxygen in the atmosphere,  $C_I$  is the concentration of oxygen in the interior of the egg, and  $H$  is the Henry's law constant describing the effective equilibrium partitioning of oxygen between the bulk atmosphere and the liquid interior of the egg. Expressing the mass-transfer in terms of ambient partial pressure of oxygen is most convenient since it is a directly controlled experimental parameter. Further, because of the linear relationship between concentration and partial pressure assumed by Henry's law, concentration can be expressed in terms of an equivalent partial pressure, i.e.,  $P_{O_2}^{\text{egg}} = HC_I$ . Finally, the Henry's law constant is not dimensionless. It is most commonly used in the form expressed above, relating partial pressure of a species in the vapor phase to its concentration in the liquid phase (see Smith et al., 2005).

The rate of oxygen consumption by the growing embryo is assumed to follow Michaelis–Menten kinetics for some rate-limiting step, given by the expression:

$$R_r = \frac{k_r C_I V}{K_M + C_I}, \quad (2)$$

where  $k_r$  and  $K_M$  are rate constants. At steady-state the rate of mass-transfer of oxygen must be balanced by its consumption by reaction, or

$$k_m A (P_{O_2} - HC_I) = \frac{k_r C_I V}{K_M + C_I}. \quad (3)$$

This equation is quadratic in  $C_I$ . It is convenient to express this equation non-dimensionally by introducing the dimensionless concentration  $\phi = HC_I/P_{O_2}$ , where  $\phi$  ranges from zero (total oxygen starvation of the embryo)

to unity (complete equilibrium or infinitely fast mass-transfer with the ambient atmosphere). Substituting this variable and collecting terms gives the dimensionless equation

$$\varphi^2 + (\alpha + \beta - 1)\varphi - \alpha = 0, \tag{4}$$

where the dimensionless constants  $\alpha$  and  $\beta$  are defined by

$$\alpha = \frac{K_M H}{P_{O_2}} \tag{5}$$

$$K_2 = K_M H = \alpha P_{O_2}, \tag{11}$$

and

$$K_3 = \frac{r_b}{k_r V}. \tag{12}$$

Finally, for fitting to experimental data correlating development time and oxygen pressure, it is convenient to rewrite Eq. (7) in a form that explicitly reveals the dependence of  $\varphi$  on the partial pressure of oxygen in the ambient atmosphere. For this case, it becomes.

$$\varphi = \frac{-((K_2/P_{O_2}) + (K_4/P_{O_2}) - 1) + \sqrt{((K_2/P_{O_2}) + (K_4/P_{O_2}) - 1)^2 + 4(K_2/P_{O_2})}}{2}, \tag{13}$$

and

$$\beta = \frac{k_r V}{k_m A P_{O_2}}. \tag{6}$$

The parameter  $\alpha$  represents the degree of saturation of the reaction with respect to oxygen:  $\alpha \ll 1$  corresponds to a completely saturated reaction rate, effectively zeroth-order with respect to oxygen concentration, and  $\alpha \gg 1$  corresponds to an unsaturated, effectively first-order reaction with respect to oxygen concentration. The parameter  $\beta$  is a measure of the relative rates of reaction to mass-transfer: for  $\beta \ll 1$ , the development of the embryo is limited by the rate of the reaction and for  $\beta \gg 1$ , development is limited by the rate of mass-transfer. The dimensionless concentration of oxygen in the interior of the egg is given by the solution of Eq. (4) with the quadratic equation, namely

$$\varphi = \frac{-(\alpha + \beta - 1) + \sqrt{(\alpha + \beta - 1)^2 + 4\alpha}}{2}, \tag{7}$$

where only the non-negative root is selected.

Now, the development time  $\tau_D$  of the egg is assumed to be inversely proportional to the rate of oxygen consumption  $R_r$  less base oxygen consumption  $r_b$  for maintenance of the egg. That is,

$$\tau_D = \frac{\kappa}{R_r - r_b}, \tag{8}$$

where  $\kappa$  is some unknown dimensionless stoichiometric constant that expresses how many units of development occur per unit of oxygen consumed. Expressing  $R_r$  with Eq. (2) and non-dimensionalizing the right-hand-side of Eq. (8), the development time is given by the expression

$$\tau_D = \frac{K_1((K_2/P_{O_2}) + \varphi)}{(1 - K_3)\varphi - K_3}, \tag{9}$$

where

$$K_1 = \frac{\kappa}{k_r V}, \tag{10}$$

where

$$K_4 = \frac{k_r V}{k_m A}. \tag{14}$$

Thus, Eqs. (9)–(14) parameterize development time and partial pressure of oxygen in the ambient air. It is clear that four constants,  $K_1, \dots, K_4$  are necessary to fit the data. In addition, it is convenient to define a fifth constant,  $K_5 = (K_1 K_4)^{-1} = k_m A / \kappa$ , which can be thought of as a scaled eggshell conductance.

A non linear least-squares fit to previously published development time data (Woods and Hill, 2004) was performed to determine the constants. The results of the fit are summarized in Table 1, and theoretical predictions compared to the experimental observations in Fig. 1. The fit is clearly very good (all  $R^2 > 0.99$ ). A few details regarding the values of constants are worth pointing out. First, the base oxygen consumption as represented non-dimensionally by  $K_3$  is very small, and practically negligible. Thus, although all eggs appear to die at  $P_{O_2} < 9$  kPa (Woods and Hill, 2004), the model here predicts that the development time of the egg will increase to infinity as the oxygen partial pressure vanishes.

Second, the value of  $\beta = K_4/P_{O_2}$  ranges from 0.36 to 1.2, with the larger values at lower partial pressures of oxygen. This indicates that mass-transfer of oxygen to the egg contributes to development time, especially at low partial pressures. Indeed, the concentration of oxygen in the egg is low ( $P_{O_2} < 2$  kPa as measured with a microelectrode, Woods and Hill, 2004), and the value of its dimensionless concentration  $\varphi$  can be approximated by

$$\varphi \approx \frac{\alpha}{\beta - 1}. \tag{15}$$

At these low concentrations, the development time is then approximated by

$$\tau_D \approx K_1 \beta = \frac{\kappa}{k_m A} \frac{1}{P_{O_2}}, \tag{16}$$

which shows clearly that mass-transfer entirely determines the development time.

Table 1  
 Constants  $K_1, \dots, K_4$  determined by a non linear, least-squares fit of Eq. (9) to the experimental data in Fig. 1

Temperature ( $^{\circ}\text{C}$ )	$K_1$ (h)	$K_2$ (kPa $\text{O}_2$ )	$K_3$	$K_4$ (kPa $\text{O}_2$ )	$K_5$ (mol $\text{O}_2$ /kPa $\text{O}_2$ – hr)	$R^2$ of model fit
22	136	0.244	$5.52 \times 10^{-5}$	10.82	$6.8 \times 10^{-4a}$	0.997
27	85	0.444	$9.00 \times 10^{-5}$	13.42	$8.8 \times 10^{-4}$	0.999
32	67	0.644	$8.94 \times 10^{-5}$	11.98	$12.4 \times 10^{-4}$	0.992

The constant  $K_5 = (K_1 K_4)^{-1} = k_m A / \kappa$ .

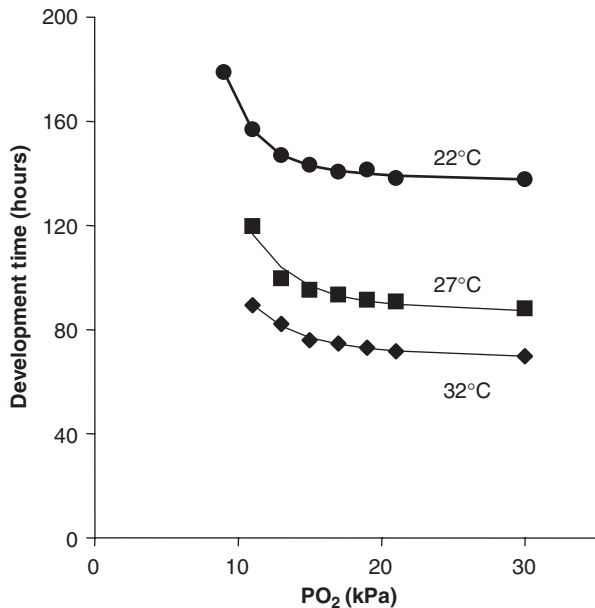


Fig. 1. Development time versus ambient oxygen partial pressure for eggs of *Manduca sexta*. Solid symbols are experimental observations from Woods and Hill (2004, Fig. 1B) and lines represent fits to the model (Eq. (9), constants listed in Table 1).

The rate constants  $K_1^{-1}$ ,  $K_2$  and  $K_5$  at the three temperatures fit well to Arrhenius expressions, which are listed in Table 1. The activation energy for the reaction rate ( $K_1^{-1}$ ) is about 59.8 kJ/mol, and the activation energy for mass-transfer ( $K_5$ ) is 44.8 kJ/mol. As usual the activation energy for reaction is greater than the activation energy for mass-transfer, but not hugely so.

The fit shows a transition from reaction-limited to mass-transfer-limited development with decreasing  $P_{\text{O}_2}$ . The data used to parameterize the model, however, used only three temperatures. To test the conclusion that eggs are designed on a temperature-dependent transition point between reaction and diffusion limitation, we used a larger range of temperatures with finer increments.

### 3. Material and methods

#### 3.1. Animals

Eggs were derived from a laboratory colony of *M. sexta*, exposed to a 14L:10D photoperiod. Eggs, larvae, and pupae were kept at 25  $^{\circ}\text{C}$  and adults at 24  $^{\circ}\text{C}$ . Adults were

given 30% honey water and potted tobacco plants for oviposition.

#### 3.2. Aluminum bar experiment

Eggs were exposed to different, constant temperatures from day 2 to hatching. For comparison, total development times (laying to hatching) measured in a previously published experiment (Woods and Hill, 2004) ranged from 70 h at 32  $^{\circ}\text{C}$  to 140 h at 22  $^{\circ}\text{C}$ .

Temperatures were imposed by placing eggs into an aluminum thermal gradient bar (Fig. 2) (custom built by the Department of Chemistry's Machine Shop at The University of Texas). To facilitate video imaging (see below), the surface of the bar was spray-painted dull black. The ends of the bar were fixed at 34 and 22  $^{\circ}\text{C}$ , respectively, by circulating water from constant temperatures baths through chambers milled in the end. Each of 12 evenly spaced rows 2.54 cm (1 in) apart of the bar contained eight 0.48 cm (3/16 in) diameter chambers drilled through the bar. Eggs were supported  $\sim 1.3$  cm (1/2 in) below the bar's surface by small pieces of cotton pushed into the wells. We used all 96 wells for eggs (1 egg well $^{-1}$ ), and into four of the wells (top wells of rows 1, 4, 9, and 12) we also placed T-type thermocouples. Temperatures were noted twice per day.

Eggs in the aluminum bar were exposed to ambient room air. This design had two consequences. First, eggs were always exposed to normoxia (21 kPa oxygen). Although we did not directly monitor oxygen levels adjacent to eggs, a brief calculation shows that oxygen levels at egg level would be indistinguishable from ambient. Imagine a well as a single giant aeropyle. The oxygen concentration at the bottom of the tube (in this case at the egg's surface) is given by a rearrangement of Fick's equation,  $C_{\text{egg}} = C_{\text{air}} - Jl/AD$ , where  $C_{\text{air}}$  is the concentration of oxygen in ambient air (8.43 mol m $^{-3}$  at 30  $^{\circ}\text{C}$ ),  $J$  is the metabolic rate of a single egg (3.3–10 mol egg $^{-1}$  s $^{-1}$  between 22 and 32  $^{\circ}\text{C}$ ; Woods and Hill, 2004),  $l$  is the length of the tube (.013 m),  $A$  is the cross-sectional area of the tube ( $1.8 \times 10^{-5}$  m $^2$ ), and  $D$  is the diffusion coefficient of oxygen in air ( $20.3 \times 10^{-6}$  and  $21.5 \times 10^{-6}$  m $^2$  s $^{-1}$  at 20 and 30  $^{\circ}\text{C}$ , respectively). Substituting these values into the equation above shows that at no biological temperature could the oxygen concentration at the egg's surface have differed from ambient by more than 0.05%.

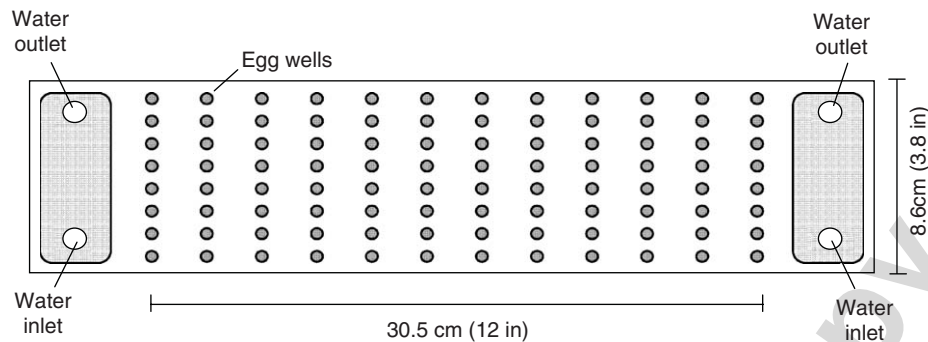


Fig. 2. Schematic of aluminum thermal gradient bar. The bar was 2.5 cm thick (1 in). To minimize heat loss from the bar's faces, it was placed into molded insulation such that only the upper surface was exposed to air.

A second issue was relative humidity, which was uncontrolled during the experiment but generally ranged between 30% and 40% (at room temperature,  $\sim 23^\circ\text{C}$ ) during the experiment. This range of relative humidities corresponds to a vapor density of about  $7\text{ g m}^{-3}$ . Because warmer air has a higher vapor capacity, eggs at the warm end of the aluminum bar were exposed to lower relative humidities (but the same vapor density). However, the change was slight—a vapor density of  $7\text{ g m}^{-3}$  corresponds to  $\sim 20\%$  relative humidity at  $32^\circ\text{C}$ —and was therefore ignored.

Oviposition times were determined to within 4 h by introducing potted tobacco plants into the adult flight cage and removing them 4 h later. Eggs were stripped from the leaves and kept for 24 h at room temperature ( $\sim 21^\circ\text{C}$ ). Subsequently, eggs were weighed individually on a microbalance (Sartorius MC5, Goettingen, Germany,  $\pm 1\mu\text{g}$ ) and assigned randomly to positions in the bar.

Two digital video cameras (Hitachi KP-D50, Tokyo, Japan,  $1/2''$  CCD,  $768 \times 494$  pixels) with magnifying lenses (Samsung 604CN, South Korea, 6–12 mm vari-focal zoom lens) were mounted above the aluminum bar and adjusted so that eggs in all wells were visible. The experiment was carried out under constant illumination so that larvae would be visible immediately when they hatched. Images from each camera were captured at 6-min (first 3 days) or 12-min (days 4–6) intervals using a frame grabber (Model 3153, Data Translation, Marlboro, MA, USA) controlled by image processing software (Global Lab Image/2 V3.0, Data Translation). Images were written to a hard drive, assembled into movies, and analysed for hatching time.

Statistical analysis was done using S-Plus v. 6.1 (Insightful, Seattle, WA, USA). Data on bar temperatures were pooled across days and analysed by least-squares linear regression. Developmental times were analysed two ways. First, total development time (from laying to hatching) was analysed as a function of temperature, with a summary description provided by fitting (least squares) a third-order polynomial to the data. Second, they were visualized as an Arrhenius plot, with rates calculated as the reciprocal of total development time versus inverse absolute temperature. Throughout, error bars refer to standard deviations.

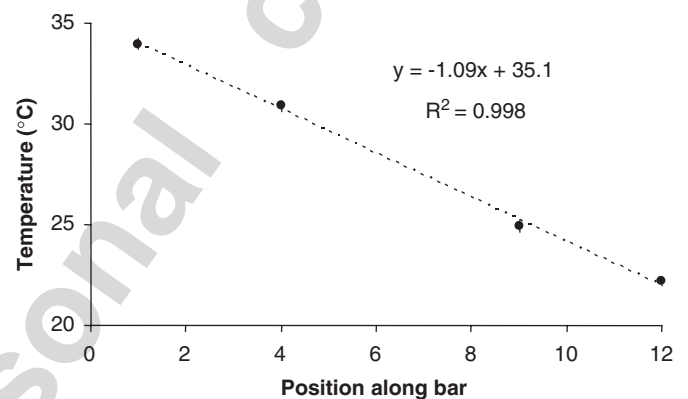


Fig. 3. Temperature gradient generated by thermal gradient bar. Each point represents the mean  $\pm$  S.D. over the six days of the experiment. The line was fitted to the data by least-squares regression.

#### 4. Results

The aluminum thermal gradient bar produced a stable, linear temperature gradient, from  $34.0 \pm 0.29^\circ\text{C}$  (mean  $\pm$  S.D.) at the hot end to  $22.0 \pm 0.23^\circ\text{C}$  at the cold end (Fig. 3), or  $\sim 1.09^\circ\text{C}$  per row of wells. Additional measurements (not shown) indicated that temperature within a row varied  $< 0.1^\circ\text{C}$  from well to well.

No eggs hatched in the two hottest rows ( $34.0$  and  $32.9^\circ\text{C}$ ), though subsequent observations suggested that embryos in these rows completed most of development before dying (pharate first-instar larva visible through the clear eggshell). In each of the remaining rows, 4–8 eggs hatched. Most of those that did not hatch ( $14/80 = 17.5\%$ ) were sterile, showing no signs of development (remained dark green). Only 2 of 80 eggs ( $2.5\%$ ) developed but did not hatch. Non-hatching eggs were discarded from further analysis.

The 64 remaining eggs had an average fresh mass ( $\pm$  S.D.) of  $1.43 \pm 0.08\text{ mg}$ . At  $31.8^\circ\text{C}$ , the warmest temperature at which hatching occurred, eggs developed in about half the time ( $3.2\text{ d} \pm 0.15$ ) that they required at  $22.0^\circ\text{C}$  ( $6.4\text{ d} \pm 0.15$ ) (Fig. 4). The data were described well ( $R^2 = 0.997$ ) by a third-order polynomial ( $y = -0.0027x^3 + 0.256x^2 - 8.27x + 92.7$ ).

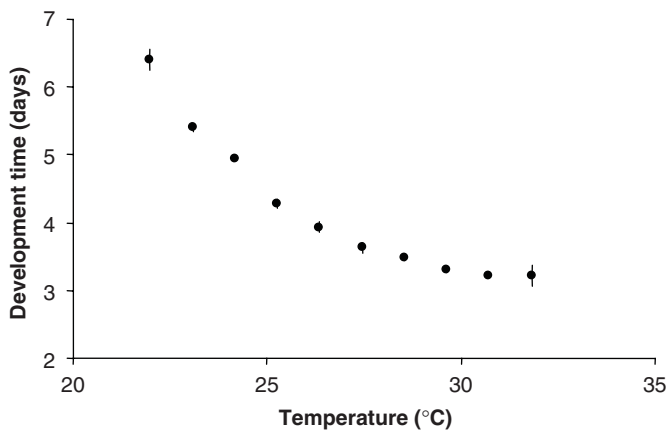


Fig. 4. Development time of eggs (of *Manduca sexta*) in the thermal gradient bar. All eggs were kept at room temperature (21 °C) for the first 24 h, then placed into the gradient bar until hatching. There are ten data points rather than 12 (number of rows in the bar) because no eggs hatched in the two warmest rows (34 and 32.9 °C).

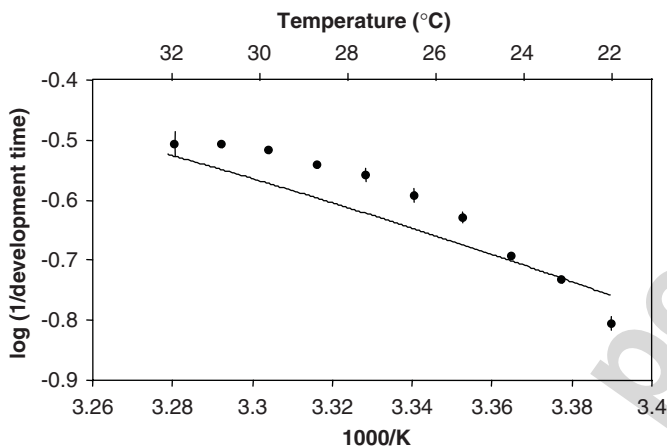


Fig. 5. Arrhenius plot of developmental data shown in Fig. 4. The curve (solid line) represents the relationship computed from the fitted model (coefficients in Table 2).

An Arrhenius plot (Fig. 5) revealed systematic but modest changes in slope across temperatures—shallow at warm temperatures and steep at cool temperatures. In Arrhenius plots, the slope of the relationship between  $\log(\text{rate process})$  and inverse temperature estimates the activation energy ( $E_a$ ) of the rate process (specifically, the slope is  $-E_a/R$ , where  $E_a$  is in  $\text{kJ mol}^{-1}$  and  $R$  is the universal gas constant,  $0.008314 \text{ kJ mol}^{-1} \text{ K}^{-1}$ ). A linear least-squares fit to the first four (warm temperatures) and last four points (cool temperatures) indicated that  $E_a$  was 8.19 and  $37.9 \text{ kJ mol}^{-1}$ , respectively.

The inverse development time versus inverse temperature can also be computed based on the model (fitted parameters in Table 2), and these results are also shown in Fig. 5. Note that in the model here, it was assumed that the eggs developed for one day at 21 °C before developing at elevated temperatures. The agreement between theoretical predictions and experimental observations is reason-

Table 2

Arrhenius fit to model constants  $K_1$ ,  $K_2$  and  $K_3$

$$K_1^{-1}(\text{h}) = 2 \times 10^7 e^{-6381/T(\text{K})}$$

$$K_2(\text{kPa O}_2) = 2 \times 10^{12} e^{-8764/T(\text{K})}$$

$$K_3(\text{mol O}_2/\text{kPa O}_2 - \text{h}) = 58420 e^{-5383/T(\text{K})}$$

The units for temperature are Kelvin.

ably good ( $R^2 = 0.70$ ). The theoretical predictions do not show as much of a variation in the slope as seen in the experimental data. This is not surprising considering that the fitted activation energies for mass-transfer and reaction rates differ by only about 50%. The experimental results seem to show a much more dramatic variation, with an approximately four-fold difference between the two activation energies.

## 5. Discussion

For aerobic biological structures relying on diffusive oxygen supply, metabolic rate will, in the extreme, be limited by either of two processes: the rate of oxygen supply or the rate at which supplied oxygen is consumed. The former is *transfer limitation* and the latter *reaction limitation*. Metabolism in most biological structures likely is limited by both processes—i.e., with omnipotent experimental powers, one could stimulate metabolic rate by increasing either diffusive oxygen supply or the quantity or concentration of enzymes and other reactants. For such biological structures, the interesting questions are: What are the relative strengths of transfer and reaction limitation? And how do the relative strengths change across environments?

The model above analyses these questions in terms of specific expressions for oxygen transfer and consumption. Mass transfer is modeled as Fickian diffusion and consumption as a saturating reaction with Michaelis–Menten kinetics. After non-dimensionalization, the steady-state equation contained just four parameters, which we fitted to previously published data (from Woods and Hill, 2004) on development time of eggs of an insect, *M. sexta*, subjected to different combinations of temperature and ambient oxygen (factorial design with 3 temperatures  $\times$  8 oxygen levels). The parameter  $\beta$ , which provides a measure of the relative rates of reaction to mass-transfer, took on values ranging from 0.36 to 1.2, indicating that eggs of *M. sexta* develop at the transition between reaction and transfer limitation.

To test this result with a new data set, we measured total development time of eggs subjected to finely-incremented temperatures between 22 and 34 °C. The rationale for the experiment was that increasing temperature usually has a more pronounced effect on rates of biochemical reaction than on rates of mass transfer by diffusion (Raven and Geider, 1988; Snyder, 1908, 1911; Woods, 1999). Raven thus, eggs at higher temperatures should show a greater

degree of limitation by transfer. This effect will appear in an Arrhenius plot as an increasingly shallow slope at high temperatures, corresponding to increasing domination by the lower activation energy of diffusion compared to reaction. The experimental development times showed just this relationship (Fig. 5). The model predicted similar behavior, though not as dramatic as the experimental data.

### 5.1. Why are structures as small as eggs of *M. sexta* ever mass-transfer limited?

The answer likely involves physical constraints on producing biological structures that prevent water efflux while allowing adequate oxygen influx, a difficulty that stems from the molecules' relative sizes. Barriers that permit passage of oxygen (molecular diameter  $\sim 3 \text{ \AA}$ ) will also allow passage of water vapor (molecular diameter  $\sim 2.8 \text{ \AA}$ ). Consequently, eggs are subject to a tradeoff. High eggshell conductance provides sufficient oxygen under diverse environmental conditions, but at the risk of desiccation. Low eggshell conductance protects water reserves, but at the cost of obtaining oxygen slowly.

This tradeoff has been studied in the context of avian eggs by Rahn, Ar, Paganelli and colleagues. They found that, across a 2000-fold range of bird egg masses, eggshell conductance to water vapor scaled to egg mass with an exponent of 0.81 (Ar and Rahn, 1978), a value close to the exponent (0.71–0.77) describing the scaling of metabolic rate to egg mass (Hoyt and Rahn, 1980; Rahn et al., 1974). This close correspondence was hypothesized to stem from the following considerations: (i) eggshells must have certain minimum conductance to guarantee sufficient oxygen supply late in development; (ii) diffusive transport of both water vapor and oxygen occurs through air-filled pores crossing the shell; (iii) the pores provide the main resistance to transport; and (iv) to conserve water, selection minimizes shell conductance as much as possible without causing severe oxygen stress. Subsequent direct measures of the conductance of chicken eggshells to water vapor and oxygen, and independent measures of  $P_{O_2}$  inside the egg, supported these ideas (Paganelli et al., 1978).

Together these results imply a physical tradeoff—mediated by the air-filled pores crossing the shell—between ability to acquire oxygen and propensity to lose water. We propose, by analogy, that selection for water conservation has driven the conductance of *insect* eggshells low enough that they lie at the edge of oxygen limitation. A significant functional difference between insect and bird eggs is the location and nature of the primary flux-resisting layer. In eggs of *M. sexta*, it occurs not in the air-filled pores crossing the outermost layer (as it does in bird eggshells) but in solid and semi-solid layers to the interior (Woods et al., 2005). Consequently, in insects the ratio of eggshell conductances to oxygen and water vapor will reflect the diffusivity ratio in liquids and solids—with possible further modification by differential chemical interactions between the diffusing gases and their medium.

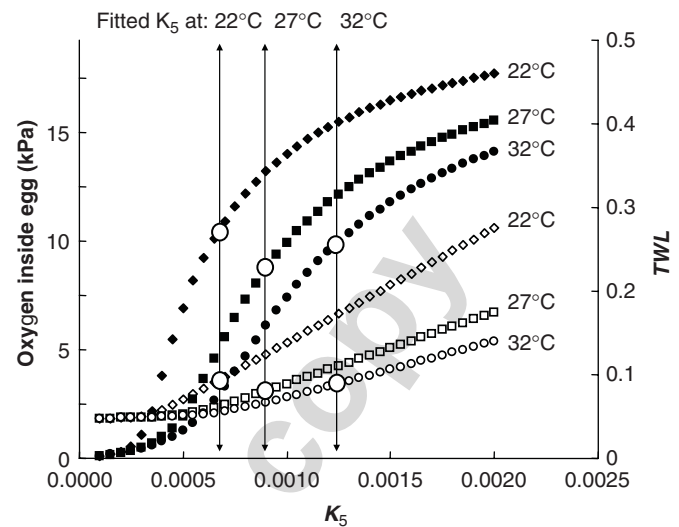


Fig. 6. Predicted concentrations of oxygen in the interior of the egg (filled symbols) and total water loss (unfilled symbols) as functions of scaled eggshell conductance,  $K_5$ . Vertical lines indicate fitted  $K_5$ 's at three different temperatures.

Empirical evidence of two kinds indicates that an oxygen–water tradeoff is real and pressing for *Manduca* eggs. First, Woods et al. (2005) measured developmental changes in metabolism and water loss in batches of *M. sexta* eggs, using a flow-through respirometry system. From laying to hatching, metabolic rate (as  $CO_2$  emission) and water loss rate increased in parallel, both rising  $\sim 3$ -fold. [We note that Fig. 6A in Woods et al. (2005) contains an error; the left axis should read ‘Water vapour flux  $\times 10^5$  ( $\text{mols}^{-1} \text{m}^{-2}$ )’]. These correlative data suggest that as embryonic oxygen demand rises, eggshell conductance increases to accommodate larger oxygen fluxes—and the associated cost is greater water efflux. Second, Zrubek and Woods (2006) recently tested the tradeoff hypothesis experimentally by subjecting 3-d old eggs of *M. sexta* to 15%, 21% (normoxia), and 35% oxygen for 24 h while measuring rates of metabolism (as  $CO_2$  emission) and water loss. Hypoxia depressed egg metabolic rates but led to pronounced, rapid increases in water loss. By contrast, hyperoxia had no significant effect on metabolism or water loss. These data demonstrate that insect eggs are active participants in balancing oxygen gain and water loss, and they constitute direct empirical support for the tradeoff hypothesis. Specifically, it appears that to allow sufficient oxygen influx under hypoxia eggs must increase eggshell conductance, which allows greater water efflux.

### 5.2. Modeling oxygen–water tradeoffs

The existence of a tradeoff suggests an optimality question: what conductance should an eggshell have? An interesting analysis of this problem has been developed in the context of bird eggshells, by Alexander (1996). He asserts, reasonably, that an egg is harmed either by losing a large fraction ( $a$ ) of its initial mass to evaporation or by

experiencing large partial pressure gradients of oxygen ( $\Delta P_{O_2}$ ) from the environment to the embryo (corresponding to low internal  $P_{O_2}$ ). If so, natural selection is likely to minimize the function

$$\Phi = a + K\Delta P_{O_2}, \quad (17)$$

where  $K$  (representing a weighting parameter that describes the relative importance of oxygen and water) is unknown but assumed not to vary among species. By replacing the

$$\varphi = \frac{-((K_2/P_{O_2}) + (1/P_{O_2}K_1K_5) - 1) + \sqrt{((K_2/P_{O_2}) + (1/P_{O_2}K_1K_5) - 1)^2 + 4(K_2/P_{O_2})}}{2}, \quad (20)$$

right-hand terms with specific descriptions of how they depend on egg mass, metabolism, incubation time, and eggshell conductance, it is possible to differentiate and find a simple equation for the conductance ( $G_{opt}$ ) that minimizes  $\Phi$ . Because  $K$  is unknown, one cannot calculate  $G_{opt}$  for any particular egg. But it is straightforward to show (Alexander, 1996) that  $G_{opt}$  should scale as  $mass^{0.78}$ —an exponent indistinguishable from the one (0.81) determined empirically by Ar and Rahn (1978).

Below we develop an alternative model, which differs from Alexander's in that it is derived explicitly from parameterized equations describing how development depends on mass transfer and consumption of oxygen (Eqs. (9)–(14)).

Because the fluxes of water and oxygen across the insect eggshell are controlled by the same sets of structures, total water lost ( $TWL$ ) will be proportional to the mass-transfer coefficient multiplied by the total developmental time, or

$$TWL \sim k_m A \tau_D. \quad (18)$$

This formula assumes that eggshell conductance is constant over development. We know that for *Manduca* this is not so—both developmental stage and current environmental conditions affect eggshell conductance (Woods et al., 2005; Zrubek and Woods, 2006). Eq. 18 therefore equates an integrated parameter ( $TWL$ ) with a nominally instantaneous one ( $k_m$ ). We still think, however, that  $TWL$  is a reasonable 'controlling' parameter. First, whether or not the egg produces a viable hatchling will depend on water reserves left near the end of development, not the instantaneous rate of water loss at any moment. That is,  $TWL$ , is likely to be more closely related to fitness (and thus under selection) than is the mass-transfer coefficient,  $k_m$ . Second,  $k_m$  at any one point may indeed constitute a good statistical predictor of  $TWL$ , because  $k_m$  in general rises over development (if  $k_m$  is high now, it will go only higher) and because environmental conditions often are autocorrelated over time scales relevant to egg development (days). Third, the fitted value of  $k_m$  (determined from developmental data) is, in fact, time-averaged, as development occurs over several days.

Now, noting that  $k_m A \sim K_5$  and substituting Eq. 9 for the total developmental time,  $\tau_D$  (and neglecting  $K_3$ , which is  $\ll 1$ ), total water loss is given by

$$TWL \sim \frac{K_5 K_1 ((K_2/P_{O_2}) + \varphi)}{\varphi}. \quad (19)$$

The dimensionless (fractional) concentration of oxygen inside the egg,  $\varphi$ , can also be rewritten as a function of  $K_5$  by substituting  $K_4 = (K_1 K_5)^{-1}$  into Eq. (13), giving

Finally, we can transform  $\varphi$  into the egg's internal partial pressure of oxygen (in kPa) by multiplying it by atmospheric  $P_{O_2}$  (assumed to be 21 kPa).

A plot of  $TWL$  and internal  $P_{O_2}$  (denoted hereafter as  $P_{O_2}^{egg}$ ) together as functions of  $K_5$  (Fig. 6) supports an interesting view of how insect eggs balance oxygen gain and water loss. We describe this view in the section below. First, though, we describe how different features of the model lead to this broader view. Note that the values of  $TWL$  are reported on an arbitrary scale since the actual constants for the equality in Eq. (19) are not known. Comparison of relative values of  $TWL$  for different values of  $K_5$  is the most important function of this plot.

The model indicates that the equivalent partial pressure of oxygen inside the egg ( $P_{O_2}^{egg} = HC_I$ ) increases monotonically from 0 to 21 kPa (assumed atmospheric  $P_{O_2}$ ) with increasing  $K_5$ . The S-shape of these curves reflects coupling of reaction kinetics and diffusive transport of oxygen. At large  $K_5$  (rapid mass transfer), oxygen moves easily into the egg and metabolic reactions are saturated (and total development time invariant) regardless of variation in  $K_5$ . However, as  $K_5$  declines below  $\sim 0.0015$ , metabolic reactions increasingly draw down internal oxygen stores, because oxygen moves less easily into the egg. Finally, at  $K_5$  below  $\sim 0.005$  (depending on temperature), metabolic reactions draw oxygen stores to very low levels—as  $K_5$  goes to zero, so too do  $P_{O_2}^{egg}$  and metabolic rate.

The  $TWL$  curves, by contrast, transition from a flat plateau at low  $K_5$  to linearly increasing  $TWL$  at large  $K_5$ . In the linearly increasing phase metabolic reactions are saturated, and, therefore, increasing  $K_5$  leads to higher rates of water loss without also shortening total development time ( $\tau_D$ ). The plateau at low  $K_5$  arises from interplay of eggshell conductance and total development time. As  $K_5$  declines, water loss *per unit time* also declines. But the rate of oxygen influx per unit time also decreases, leading to longer development times over which water loss occurs. A priori we had expected that  $TWL$  would increase at low  $K_5$ , because development would stall completely while water would continue to be lost. This result did not occur because the fitted values of  $K_3$  (representing oxygen consumption

devoted only to maintenance, not development) were negligibly small.

Temperature affects both  $P_{O_2}^{egg}$  and  $TWL$ . Increasing temperature depresses the curves of  $P_{O_2}^{egg}$ , because the activation energy of metabolism is larger than that of mass transport—i.e., as temperature rises, metabolic consumption of oxygen increasingly outstrips diffusive delivery of oxygen. However, this effect is mostly offset by increasing eggshell conductance ( $K_5$ ) at higher temperatures. The net effect (seen where the vertical lines, representing fitted values of  $K_5$ , cross the curves of  $P_{O_2}^{egg}$ ) is that  $P_{O_2}^{egg}$  is predicted not to change much at different temperatures. Using an oxygen microelectrode, Woods et al. (2005) measured internal  $P_{O_2}^{egg}$  in ambient air at 24 and 37 °C, finding that it was slightly but significantly lower at the higher temperature. We note, however, that the theoretical analysis extends only to 32 °C whereas the electrode data were from 37 °C. Also, the model also assumes an average oxygen demand throughout development. Indeed the demand for oxygen increases with time as the number of living cells increases over development. This would lead to a greater-than-predicted decrease in the oxygen concentration toward the end of development.

Counter-intuitively, the model also predicts that at a given  $K_5$  increasing temperature depresses  $TWL$ . This phenomenon occurs because temperature's effect on shortening total development time more than offsets its direct stimulation of water loss rate, at least in the range between 22 and 32 °C. Note again, though, that fitted value of  $K_5$  increases with temperature—such that the predicted  $TWL$  between 22 and 32 °C remains approximately constant. Clearly, long-term exposure of eggs to temperatures above 32 °C leads to death under our standard laboratory conditions. For shorter time periods, wild eggs in many parts of *M. sexta*'s range (Ferguson et al., 1999) Ferguson et al. regularly experience temperatures above 32 °C. What happens in this temperature range is a key to predicting evolutionary pressures on different populations: higher temperatures stimulate higher rates of water loss, but whether they also stimulate  $TWL$  will depend on whether exposure to brief high temperatures accelerates development, has no effect, or slows development.

### 5.3. How insect eggs balance oxygen gain and water loss

Terrestrial eggs of insects cannot acquire oxygen without losing water (see Zrubek and Woods, 2006). Given this tradeoff, what is the best eggshell conductance to have? Our analysis indicates that conductance of the *M. sexta* eggshell is low enough such that internal  $P_{O_2}^{egg}$  is near the  $K_M$  for whole-egg oxygen use. Conductance could be lower, which would result in water savings but would also cause a steep decline in  $P_{O_2}^{egg}$  and increase total development time ( $\tau_D$ ). In an optimal foraging context (eggs 'foraging' for oxygen), eggshell conductance appears to be set high enough to have exploited the steeply increasing part of the curve of  $P_{O_2}^{egg}$ . Above the fitted values of  $K_5$ , the

marginal value to be gained from still higher conductance becomes increasingly small.

The fitted model parameters and graphical analysis shown in Fig. 6 are specific to *M. sexta*. However, the general conclusions should be applicable to eggs of most terrestrial organisms. All terrestrial eggs face the twin pressures of having to limit water loss while still developing rapidly, and this situation will lead to the evolution of eggshell conductances that place embryos at risk for both desiccation and oxygen starvation. We note that two additional factors likely also shape eggshell function: the drying power of the environment (microsite temperature and humidity together) and the predation risk. The latter is particularly interesting. High predation risk should favor the evolution of higher conductances (increasing total water expenditure) as a means of minimizing total development time.

### Acknowledgments

A special thanks to Terry Watts for building the aluminum thermal gradient bar used in this project. This work was supported by the NSF (IBN-0213087 to HAW) and the University of Texas at Austin.

### References

- Alexander, R.M., 1996. Optima for Animals. Princeton University Press, Princeton, NJ.
- Ar, A., Rahn, H., 1978. Interdependence of gas conductance, incubation length, and weight of the avian egg. In: Piiper, J. (Ed.), Respiratory Function in Birds, Adult and Embryonic. Springer, Berlin.
- Chapman, L.J., Schneider, K.R., Apodaca, C., Chapman, C.A., 2004. Respiratory ecology of macroinvertebrates in a swamp-river system of east Africa. Biotropica 36, 572–585.
- Fassnacht, D., Pörtner, R., 1999. Experimental and theoretical considerations on oxygen supply for animal cell growth in fixed-bed reactors. J. Biotechnol. 72, 169–184.
- Ferguson, D.C., Harp, C.E., Opler, P.A., Peigler, R.S., Pogue, M., Powell, J.A., Smith, M.J., 1999. Moths of North America. Northern Prairie Wildlife Research Center Home Page, Jamestown, ND <http://www.npwrc.usgs.gov/resource/distr/lepid/moths/mothsusa.htm>.
- Hoyt, D.F., Rahn, H., 1980. Respiration of avian embryos—a comparative analysis. Resp. Physiol. 39, 255–264.
- Kranenbarg, S., Muller, M., Gielen, J.L.W., Verhagen, J.H.G., 2000. Physical constraints on body size in vertebrate embryos. J. Theor. Biol. 204, 113–133.
- Lamer, A., Dorn, A., 2001. The serosa of *Manduca sexta* (Insecta, Lepidoptera): ontogeny, secretory activity, structural changes, and functional considerations. Tissue Cell 33, 580–595.
- Paganelli, C.V., Ackerman, R.A., Rahn, H., 1978. The avian egg: in vivo conductances to oxygen, carbon dioxide, and water vapor in late development. In: Piiper, J. (Ed.), Respiratory Function in Birds, Adult and Embryonic. Springer, Berlin, pp. 212–218.
- Pörtner, H.O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88, 137–146.
- Pörtner, H.O., 2002. Climate variation and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp. Biochem. Physiol. 132A, 739–761.
- Rahn, H., Paganelli, C.V., Ar, A., 1974. The avian egg: air-cell gas tension, metabolism and incubation time. Resp. Physiol. 22, 297–309.

- Raven, J.A., Geider, R.J., 1988. Temperature and algal growth. *New Phytol.* 110, 441–461.
- Richardson, R.S., 2000. What governs skeletal muscle  $v_{o2max}$ ? New evidence. *Med. Sci. Sports Exer.* 32, 100–107.
- Roca, J., Hogan, M.C., Story, D., Bebout, D.E., Haab, P., Gonzalez, R., Ueno, O., Wagner, P.D., 1989. Evidence for tissue diffusion limitation of  $V_{O2max}$  in normal humans. *J. Appl. Physiol.* 67, 291–299.
- Rothschild, L. W. R., Jordan, K., 1903. A revision of the lepidopterous family Sphingidae. *Novitates Zool.* 9 Suppl.
- Smith, J.M., Van Ness, H.C., Abbott, M.M., 2005. *Introduction to Chemical Engineering Thermodynamics*, seventh ed. McGraw-Hill, New York.
- Snyder, C.D., 1908. A comparative study of the temperature coefficients of the velocities of various physiological actions. *Am. J. Physiol.* 22, 309–334.
- Snyder, C.D., 1911. On the meaning of variation in the magnitude of temperature coefficients of physiological processes. *Am. J. Physiol.* 28, 167–175.
- West, G.B., Brown, J.H., Enquist, B.J., 1997. A general model for the origin of allometric scaling laws in biology. *Science* 276, 122–126.
- Woods, H.A., 1999. Egg-mass size and cell size: effects of temperature on oxygen distribution. *Am. Zool.* 39, 244–252.
- Woods, H.A., Bonnecaze, R.T., Zrubek, B., 2005. Oxygen and water flux across eggshells of *Manduca sexta*. *J. Exp. Biol.* 208, 1297–1308.
- Woods, H.A., Hill, R.I., 2004. Temperature-dependent oxygen limitation in insect eggs. *J. Exp. Biol.* 207, 2267–2276.
- Zrubek, B., Woods, H.A., 2006. Insect eggs exert rapid control over an oxygen-water tradeoff. *Proc. Roy. Soc. B*, 273, 831–834.