Animal behaviour

Turtle embryos move to optimal thermal environments within the egg

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A recent study demonstrated that the embryos of soft-shelled turtles can reposition themselves within their eggs to exploit locally warm conditions. In this paper, we ask whether turtle embryos actively seek out optimal thermal environments for their development, as do post-hatching individuals. Specifically, (i) do reptile embryos move away from dangerously high temperatures as well as towards warm temperatures? and (ii) is such embryonic movement due to active thermoregulation, or (more simply) to passive embryonic repositioning caused by local heat-induced changes in viscosity of fluids within the egg? Our experiments with an emydid turtle (Chinemys reevesii) show that embryos avoid dangerously high temperatures by moving to cooler regions of the egg. The repositioning of embryos is an active rather than passive process: live embryos move towards a heat source, whereas dead ones do not. Overall, our results suggest that behavioural thermoregulation by turtle embryos is genuinely analogous to the thermoregulatory behaviour exhibited by post-hatching ectotherms.

1. Introduction

Thermoregulatory behaviour plays an important role in the biology of ectotherms, allowing them to maintain relatively constant body temperatures even when ambient temperatures fluctuate strongly. For example, sun-basking can enable an ectotherm to increase its body temperature quickly in the morning, and shuttling between sun and shade may allow that animal to regulate its body temperature precisely at midday [1,2]. This thermoregulatory ability can enhance fitness-relevant behavioural and physiological processes, such as locomotor capacity, food assimilation rate and reproductive output [3,4]. Behavioural thermoregulation is widespread in the post-hatching stages of ectotherms, including insects, fishes, amphibians and reptiles, but until recently it was assumed not to occur in the embryonic phase of the life cycle [1,2]. A recent study challenged this assumption by demonstrating that embryos of the Chinese soft-shelled turtle (Pelodiscus sinensis) can move within the egg to exploit warmer regions (those that are closer to the sun-heated ground surface) [5].

Potential fitness benefits of behavioural thermoregulation by turtle embryos are clear (because developmental temperatures can substantially affect developmental rates and hatching phenotypes), but whether this surprising behaviour in embryos is genuinely analogous to that in adults remains unclear. To assess the potential significance of thermoregulatory behaviour in embryonic development of oviparous reptiles, we need to answer two fundamental questions. First, do embryos reposition themselves not only to gain heat, but also to avoid thermal extremes (as do adult reptiles [1,2]). Unless embryos can move away from as well as towards ‘hot-spots’ within the egg, the analogy between embryonic repositioning and an adult reptile’s behavioural thermoregulation is weak. Second, is there a simpler physics-based explanation for embryonic repositioning, perhaps due to thermal influences on egg-fluid viscosity rather than to active movement by
embryos? The lack of overt locomotor structures in embryos suggests that embryos might simply drift towards a hot-spot because local temperatures affect the viscosity of fluids (e.g. the yolk or the amniotic fluid), causing the embryo to move passively towards regions of higher temperature. We conducted experiments on the embryos of an emydid turtle (Chinemys reevesii) to explore these two issues.

2. Material and methods

(a) Embryonic movement under different thermal regimes

We incubated eggs of the Chinese three-keeled pond turtle (C. reevesii) in various thermal environments to investigate embryonic movements, at temperatures ranging from 26°C to 33°C. This species is an aquatic emydid found in central and southern China and southeastern Asia; clutches average approximately six eggs [6]. Incubation temperatures affect developmental rate of embryos and body size, sex and locomotor performance of hatchlings in C. reevesii [7]. Most natural nests are likely to be cooler than 33°C (see the electronic supplementary material, figure S1), and eggs that are chronically exposed to temperatures above 32°C have low hatching success [7].

A total of 125 recently laid C. reevesii eggs (mean mass = 10.2 g, mean egg length = 35 mm) from a turtle farm in Zhejiang were incubated individually in 80 ml jars containing moist vermiculite (∼220 kPa), in an incubator (FPQ incubator, Ningbo Life Science and Technology Ltd, China) set at 26°C, and randomly assigned among five thermal treatments: (i) constant temperature of 26°C; (ii) dorsal heating to 29°C on the upper surface of the egg; (iii–v) lateral heating to 29°C, 30°C and 33°C, directed towards the pointed ends of eggs. The eggs were heated by 75 watt electronic heating mats (500 × 450 mm), with the distance between the jars and the heating mats adjusted to 220, 154 or 41 mm to obtain the desired egg-surface temperatures of 29°C, 30°C or 33°C (at the end of the egg that was closest to the heat source). We monitored temperatures at the ends of eggs closest to and furthest from the heat source at 30 s intervals, using 40 gauge thermocouples (TCTTT140, Temperature Controls Pty Ltd) connected to a data-taker (DT-80, Datataker Pty Ltd) to measure thermal gradients within a single egg. The thermocouples (±0.01°C) were calibrated to a standard thermometer prior to the experiment. The thermal difference between the hot and cold ends of an egg averaged 1.0°C, 1.2°C or 1.6°C for 29°C, 30°C or 33°C treatments, respectively. At the beginning of each experiment, we used candling to quantify the position of the embryo’s midpoint (defined as the point where the neck met the carapace, an obvious morphological feature in turtle embryos; figure 1), which was normally close to the midline of eggs, and marked it on the egg surface with a pencil. One week later, we quantified the position of the embryo’s midpoint again to determine the distance (± 0.01 mm) that embryos had shifted from their original position along the long axis of the egg. This measurement is highly repeatable (mean disparity between repeated measures = 1.26 mm, 95% CI = 0.94–1.59 mm; see the electronic supplementary material).

(b) Movement of live versus dead embryos

Eggs of C. reevesii were incubated individually in 80 ml jars containing moist vermiculite (∼220 kPa), exposed to lateral heating at a temperature of 28°C (optimal for embryonic development in this species [7]). After 10 days (for the turtle, the developmental stage of embryos = 18 [8]) of incubation, we injected half of the eggs (randomly selected) with urethane to euthanize the embryos. We measured the position of embryos (as above) before injecting, and one week later by candling. Changes in the embryo’s location within its egg were calculated as the distance shift of the embryo’s body in the week after treatment, along the long axis of the egg.

(c) Data analysis

We used Kruskal–Wallis ANOVA to analyse the effect of the direction of heat source on embryonic positions in turtle eggs. The post hoc Nemeyi test was used to identify significant differences in embryonic movement among treatments. The Mann–Whitney U test was used to compare the extent of repositioning of live versus dead embryos in response to a lateral heat source. In the text, data are presented as mean ± s.e. The raw data used in the analyses may be found in the Dryad data depository (http://dx.doi.org/10.5061/dryad.d35q5).

3. Results

(a) Embryonic movement under different thermal regimes

Thermal treatments affected embryonic positions in the emydid turtle C. reevesii (H₄₁₂₄ = 15.4, p < 0.01). Under constant temperature or dorsally directed heating, embryos remained near the midpoints of their eggs. Under lateral heating, the embryos moved towards a heat source generating...
References


