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Hotter nests produce smarter young lizards

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A hatchling reptile's sex, body size and shape, and locomotor performance can be influenced not only by its genes, but also by the temperature that it experiences during incubation. Can incubation temperature also affect a hatchling's cognitive skills? In the scincid lizard *Bassiana duperreyi*, higher incubation temperatures enhanced the resultant hatchling's learning performance. Hence, factors such as maternal nest-site selection and climate change affect not only the size, shape and athletic abilities of hatchling reptiles, but also their ability to learn novel tasks.

Keywords: incubation; temperature; learning; climate change

1. INTRODUCTION

An animal's developmental environment helps shape its phenotypic traits [1]. Thus, an adult's phenotype reflects not only its genetic constitution, but also the impacts of influences encountered earlier in life [2]. Indeed, the earliest developmental stages often are the most sensitive to external conditions [3]. Especially in oviparous species, where offspring develop outside the mother's body, incubation environments can have long-lasting effects [4,5]. Thermal, hydric and gas-exchange conditions all vary within [6] as well as among nests [7], generating variation among hatchlings in phenotypic traits such as sex, body size, shape and locomotor ability [5,8].

Most researchers have focused their attention on hatchling traits that are easy to measure, and that are plausibly relevant to individual fitness. As a result, we have considerable data on developmental plasticity in traits such as morphology and locomotor ability, but a very limited understanding of how incubation environments affect behavioural traits such as learning ability (but see Burger [9]). Nonetheless, learning ability is important: a growing literature suggests that greater behavioural flexibility can help individuals deal with novel challenges, and allow them to respond more successfully to environmental stochasticity [10,11]. Thus, any effect of incubation conditions on a hatchling's learning ability might have long-term consequences on that individual's viability.

Here, we examine whether incubation temperature affects lizards' ability to learn the location of a safe retreat site during a predatory attack. We focus on predator escape because it is directly relevant to individual survival and therefore fitness [12].

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2. MATERIAL AND METHODS

(a) Egg collection and incubation

We collected gravid three-lined skinks (*Bassiana duperreyi*) from the Brindabella Range, 40 km west of Canberra in the Australian Capital Territory, at elevations ranging from 1050 to 1700 m. Female lizards were transported to the University of Sydney, where they were kept in cages (22 × 13 × 7 cm) containing a substrate of moist vermiculite (water potential = -200 kPa) to lay their eggs. Newly laid eggs were weighed and transferred to individual 64 ml glass jars filled with the same vermiculite mixture (-200 kPa) and sealed with plastic cling wrap to prevent moisture loss during incubation. We tested hatchlings from 12 clutches between one and four months of age (six male and six female hot-incubated skinks and six male and six female cold-incubated lizards).

To account for maternal effects, we used a split-clutch design, randomly dividing eggs from each clutch between two incubation treatments ('hot' = diel cycle of 22 ± 7.5°C; 'cold' = diel cycle of 16 ± 7.5°C). These treatments mimic thermal regimes typical of natural nests at low (hot) versus high (cold) elevations [13]. We used four 10-step Claydon incubators (Brisbane, Queensland), two programmed for the cold treatment and two programmed for the hot treatment. Thermal conditions were verified using iButton thermocrons (Dallas, TX, USA). As soon as a lizard hatched, we recorded its snout-vent length (SVL) and sex. We used a racetrack to record locomotor speed (see Elphick & Shine [5] for detailed methods).

(b) Learning task

Reptiles are more likely to display significant learning if they are tested in a familiar environment [12]. Hence, we trained hatchlings within the same opaque plastic containers in which they were housed. The containers (64 × 41 × 21 cm) had a sand substrate, and two potential hides (inverted plastic flower-pot trays) positioned 60 cm apart. The hides were identical except that one had a Plexiglas cover over the opening, preventing ingress by the lizard. The position of the open and closed hides remained constant throughout the experiment, enabling lizards to learn the location of the open hide. Prior to the first trial, hatchlings had 24 h to acclimate to their surroundings. Water and crickets were provided ad libitum after daily testing. Room temperature was maintained at 24°C, close to the mean body temperature recorded for this species in the field [14].

Prior to trials, hatchlings were briefly transferred to holding pens (24°C), while sand in the experimental containers was mixed to ensure hatchlings did not rely on chemical cues to locate the open hide. A hatchling was placed directly between the two hides under a small plastic cover, which was then removed, and the lizard was stimulated to run by touching it on the tail with an artist's paintbrush. Lizards that located the correct retreat site within 30 s (timed from a video recording) were scored as having a successful 'escape'. Lizards that had not found the open hide within 120 s were then placed under the correct hide. Each hatchling was tested four times per day for 4 days (totally 16 trials), with at least 45 min recovery between each trial.

(c) Analyses

To compare learning ability among hatchling lizards, we created an index of standardized learning scores (total number of successful 'escapes' in the last eight trials minus the total number of successful 'escapes' in the first eight trials). A positive score shows that a lizard located the retreat site more often in later trials, indicating learning. We scored errors based on the first hide that the lizard attempted to enter. Statistical analyses were carried out using SPSS (v. 19.0.0) at a significance level of $\alpha = 0.05$. We used ANOVAs to assess differences in learning scores among hot- versus cold-incubated hatchlings and male versus female hatchlings. We used correlation analysis to examine relationships between learning scores, body size and locomotor speed. ANCOVA with incubation treatment as the factor, SVL and speed as the covariates, and learning score as the dependent variable were used to explore the possibility that the effects of incubation treatment were secondary consequences of incubation-induced differences in size or speed.

3. RESULTS

Overall, hot-incubated lizards achieved higher learning scores than did cold-incubated lizards (mean learning score = 1.58, s.e. = 0.61, versus -1.11, 0.61; $F_{1,19} = 9.36$, $p < 0.006$; effect size $r = 0.57$; figure 1) and the number of errors they made decreased more from the first to the second half of the trials than was the case for cold-incubated lizards ($F_{1,19} = 4.26$, $p = 0.05$). Female lizards had non-significantly lower

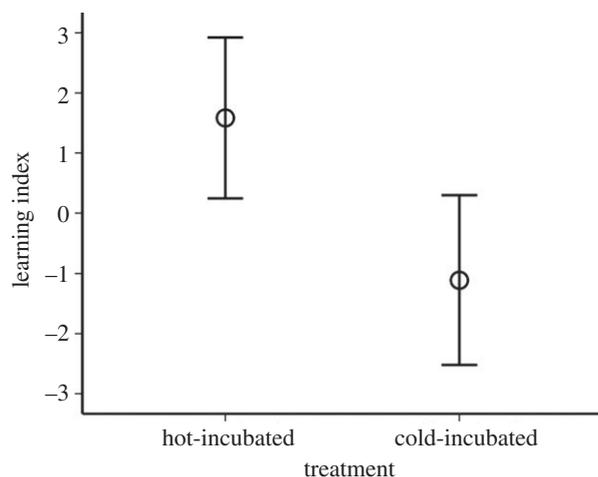


Figure 1. Mean learning scores for hatchling lizards (*Bassiana duperreyi*) incubated under hot ($22 \pm 7.5^\circ\text{C}$) and cold ($16 \pm 7.5^\circ\text{C}$) fluctuating diel temperature regimes. Error bars represent 95% CI.

average learning scores than males (0, 0.75 versus 0.75, 0.73; $F_{1,19} = 0.5$, $p = 0.49$). Incubation treatment affected learning scores similarly in males and females (interaction $F_{1,17} = 0.30$, $p = 0.59$). A hatchling's learning score was not significantly related to its SVL ($r^2 = 0.013$, $p = 0.313$) or locomotor ability ($r^2 = 0.0034$, $p = 0.409$). The incubation-treatment effect on learning score remained significant even when lizard SVL and locomotor speed were included as covariates in an ANCOVA ($F_{1,13} = 19.25$, $p = 0.001$).

4. DISCUSSION

The thermal regimes that lizards experienced during incubation affected their learning ability after hatching. This difference in learning ability does not appear to be an indirect consequence of incubation-induced shifts in other traits, because an animal's learning score was not related to its locomotor speed, body size or sex; and the incubation effect on learning remained significant even after controlling for these other effects. Thus, our study adds learning ability to a growing list of phenotypically plastic traits that incubation temperature can modify during early squamate development [5,8]. Our study was short-term, so it remains possible that such effects are transitory; that is, cold-incubated lizards eventually compensate through later development of learning abilities, or by modifying other aspects of their behaviour. For example, an intrinsically poor learner might compensate for that deficit by maintaining relatively high temperatures, thus facilitating predator escape through greater awareness and locomotor speed rather than relying on cognitive faculties, such as spatial memory. Future work could test that possibility.

What proximate mechanisms render learning sensitive to incubation temperature? Endocrine pathways can affect performance in learning tasks [15], suggesting that incubation induced modifications either to hormone levels or receptors may play some role. Especially in reptile species with temperature-dependent sex determination (TSD), such as *B. duperreyi*, thermal regimes in the nest may affect the

hormones responsible for gonadal differentiation [16,17]. These same hormones may influence brain development [17]. Thus, thermal effects on hormone levels during incubation may induce structural variation in parts of the brain that control behaviours such as learning [18].

Greater learning ability may facilitate an individual's responses to diverse environmental challenges, thus increasing its chances for survival and reproduction. Whether the effects of incubation temperature on learning ability ultimately impact individual fitness remains a challenge for future research. Our study is just a beginning. We need studies on a broad range of other taxa to determine whether it is commonly true that an individual's learning ability is modified by the incubation regimes under which it develops. We doubt that *B. duperreyi* is unique in this respect; for example, incubation temperatures affect learning ability in the honeybee, *Apis mellifera* [19]. Learning responds plastically to prenatal and postnatal developmental factors in humans [20] and rats [21]. Developmental stressors (e.g. drugs and alcohol) applied during incubation cause learning deficits in chickens [22]; however, the virtual absence of studies on other oviparous taxa precludes any statements about the generality of incubation effects on learning.

The effects of incubation temperature on learning ability may also have consequences for population-level responses. For example, anthropogenically induced shifts in air temperatures can influence nest temperatures of ectotherms—and indeed, have already affected nest thermal regimes in our study species, *B. duperreyi* [23]. Thus, climate change may simultaneously generate novel challenges for post-hatching organisms [24], while also modifying their ability to respond flexibly to such challenges. In *B. duperreyi*, hotter natural nests over recent decades (due to climate change: [23]) probably have produced hatchling lizards with enhanced learning abilities. For other species, however, the evoked plasticity may render them less rather than more capable of dealing with a changing environment. For example, some lizard taxa sympatric with *B. duperreyi* benefit from cooler rather than warmer incubation [25]. For such taxa, increasingly warm nests may generate hatchlings that are unlikely to possess the kind of behavioural flexibility needed to confront novel challenges.

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