Auburn University Journal of Undergraduate Scholarship

Isolating the Effects of Individual Nest Characteristics on Offspring Phenotypes of Brown Anole Lizards

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Abstract

The influence of the external environment on offspring phenotypes and survival is well described for a variety of oviparous (i.e., egg-laying) species. Much of this previous work has focused on replicating natural incubation environments in the laboratory, based on the characteristics of maternally chosen nest sites in the wild. Although several environmental factors affect offspring phenotypes (e.g., substrate type, soil moisture, temperature), most studies do not assess the relative contribution of each factor to variation in offspring phenotype. To understand how multiple nest characteristics interact to affect offspring phenotypic variation, we studied the brown anole lizard (*Anolis sagrei*) and evaluated three major factors that typically vary among their nest sites: substrate type, incubation temperature, and soil moisture. We show that relatively moist soil increases egg mass during development at a greater rate than other treatments. Egg incubation temperature strongly affected the developmental rate of embryos, and moisture explained most of the variation in hatchling body size. Substrate had no effect on any phenotype. We found no interactive effect among incubation substrate, moisture, or temperature on variation in hatchling morphology.

Introduction

Developmental plasticity is a phenomenon where a single genotype can express multiple phenotypes depending on their developmental environment (West-Eberhard 2003). Most organisms exhibit sensitivity to their developmental environment; therefore, conditions experienced during embryonic development can have large effects on offspring phenotypes. Oviparous organisms with little to no parental care are great study organisms for developmental plasticity because their eggs are subject to their surrounding environment for the majority of their development without any buffering due to parental care (Noble et al. 2018). Reptiles are particularly well suited for studies of developmental plasticity since eggs that are left unattended within nests are exposed to a wide range of environmental variations, including variations in temperature, moisture, and substrate type (Warner et al. 2018).

Developmental plasticity has been documented in a wide range of taxa, but many studies that examine developmental plasticity do not use ecologically relevant environmental conditions. Understanding the influence of natural conditions on the phenotype of offspring can provide insight into the variation within natural populations and how natural selection acts on that variation. Reptiles are commonly used in experiments characterizing phenotypic variation across differing temperatures (While et al. 2018). However, these studies rarely consider the effects of other nesting variables and how they interact. Natural variation in one environmental variable can have an impact on the state of another environmental variable, so understanding how these natural conditions interact is critically important. For example, both temperature and moisture conditions can affect the phenotypes of offspring (Packard & Packard 1988; Deeming 2004; Warner et al. 2012), and these two variables can potentially influence each other. Decoupling the effects of multiple variables is important for understanding the relative contributions of each variable on phenotypic development. Our goal is to take ecologically relevant nest environments and tease apart certain environmental factors (i.e., temperature, moisture, substrate) to quantify the individual effects of each variable and their interactions.

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Methods

To address our goals, we used the brown anole lizard (*Anolis sagrei*) (Fig. 1). Adult Lizards were collected from our study population at Tomoka State Park in Florida in April 2023. A breeding colony of 50 pairs was established at Auburn University. Over the summer, eggs were collected from the breeding pairs and placed in 1 of 8 different treatments. The treatments are set up in a full factorial design with two levels of temperature (hot, cold), two types of substrates (sand, soil), and two levels of moisture (wet, dry). All eggs were placed in incubators programmed to mimic hot (Mean 29.17°C, Max 40.33°C, Min 24.83°C) and cold (Mean 26.81°C, Max 29.67°C, Min 24.33°C) nest environments measured from the field. The dry and wet conditions were manipulated by placing eggs in substrates set at water potentials of either -120 kPa or -50 kPa, respectively. Incubation substrate was either sand or soil collected from our field site in an area where eggs have been previously found (Pruett et al. 2020).

Eggs were weighed when they were collected and again once every week until they hatched to record egg growth over time in the different treatments; change in mass reflects water uptake by the eggs. When the eggs hatched, snout-vent length (SVL), tail length, and mass of the hatchlings were recorded as well as their sex.

Fig. 1 Male (left) and female (right) brown anole lizard.

Results

Egg growth was significantly influenced by the moisture and temperature treatments (Fig. 2). Eggs from the hot treatment had increased growth over time compared to the cold and exhibited faster development resulting in early hatching. Eggs from the moist treatment also had increased growth over time but did not hatch earlier than those from the dry treatment.

Most of the variation in hatchling traits was influenced by incubation temperature or moisture (Fig. 3). The hot incubation treatment shortened the developmental time by about 11 days compared to the cool treatment. Hot temperatures also reduced body size, but these effects were not statistically supported. Wet incubation conditions generated relatively large offspring compared to dry conditions. The substrate had no significant effect on hatchling phenotypes, and we found no significant interactions between the three incubation variables. Male offspring had slower embryonic developmental rates than females, but the difference was only by about 1 day.

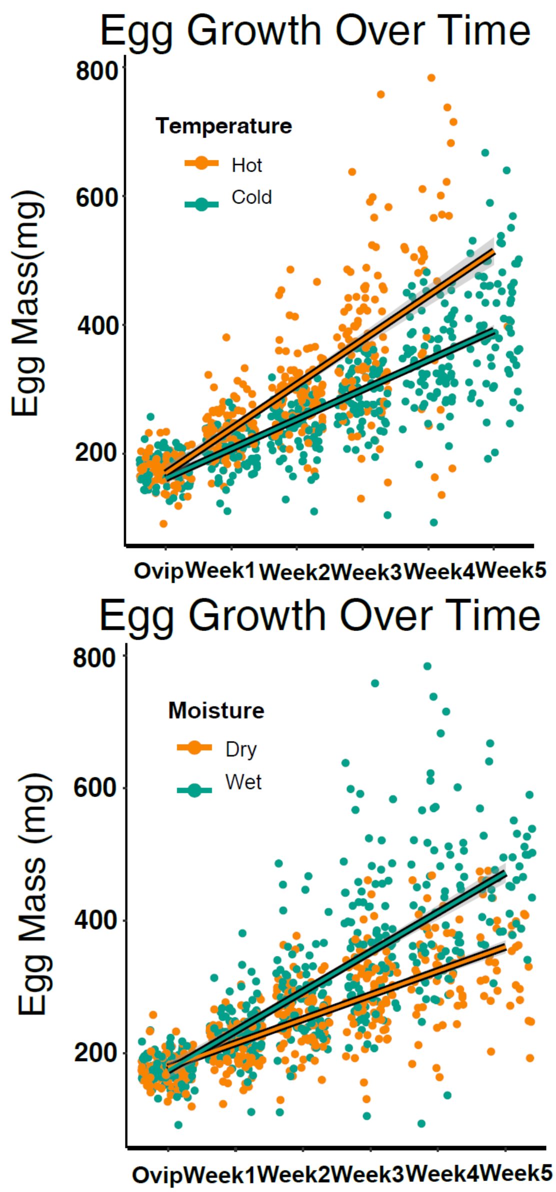

Fig. 2 Change in egg mass over the incubation period for each temperature treatment (top graph) and the two moisture treatments (bottom graph).

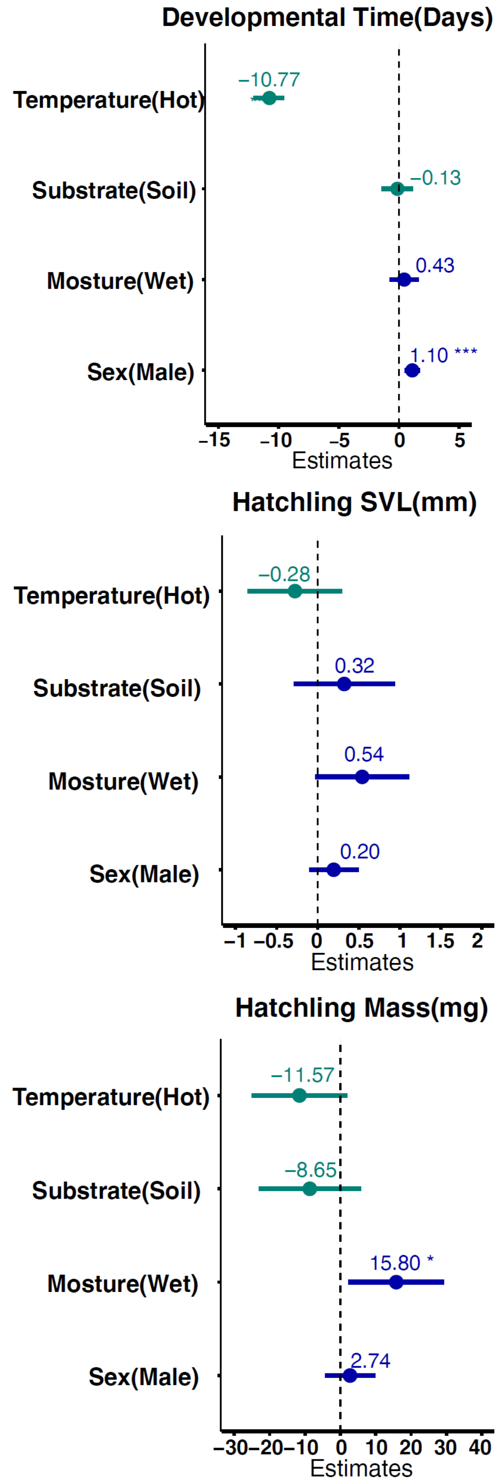

Fig. 3 The effect of temperature, substrate, moisture, and sex on developmental time (top graph), hatchling snout-vent length (SVL) (middle graph), and hatchling mass (bottom graph). Data points are effect sizes and bars represent standard errors. The treatments in parentheses are the reference treatments for calculating effect sizes. Values indicate effect sizes, and asterisks denote statistical significance: *<0.05, **<0.001, ***<0.0001.

Discussion

The nest environment that brown anole eggs experience before hatching has significant impacts on the development of the hatchlings. We show that different aspects of the natural nest environment can influence different traits of the embryos and the hatchlings. Interestingly, the effects of temperature and moisture appear to operate in isolation, at least under the conditions used in our experiment. Indeed, we found no interactive effects among temperature, moisture, and substrate type. To our knowledge, this is the first study that has simulated natural nest conditions to isolate the individual effects of these different factors.

The phenotypic effects of different components of the natural nest environment will likely have consequences on offspring fitness. Higher temperatures increase the rate of development of the embryos, thereby enabling hatchings to emerge earlier in the season compared to those experiencing cooler temperatures (Pearson & Warner 2018). Higher levels of moisture increased the mass of offspring at hatching, likely due to increased metabolic rates under relatively moist environments resulting in more yolk conversion to tissue (Miller & Packard 1992). These effects of the nesting environment on phenotypic development can have fitness consequences, and possibly have life-long impacts on their survival and future reproduction (Mitchell et al. 2018). Accelerated development and larger body size are conditions that could be beneficial in different scenarios (Delaney & Warner 2016; Pearson & Warner 2018). For instance, an organism produced early in the breeding season would have more time to grow after hatching so it may benefit from a hotter nest environment. On the other hand, offspring from late-produced eggs would emerge towards the end of the season and therefore have less time to grow and have a reduced chance of achieving a larger body size before winter. Moreover, late-hatched offspring emerge into a highly competitive environment with the early-produced cohorts. Understanding these patterns raises further questions about the effects of the behavioral choices that mothers make when selecting microhabitats for egg laying as well as other potential factors that could influence the nest environment like rainfall patterns or changes in temperature due to climate change.

Statement of Research Advisor

John Rodgers was instrumental in the early design of this experiment and helped develop the protocols for measuring different phenotypic traits of lizards. He took care of the lizard colony, collected/incubated eggs, and performed all the measurements. He was closely involved with the statistical analyses, and he wrote the early draft of this report.

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Authors Biography

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