

Original Article

Maternally chosen nest sites positively affect multiple components of offspring fitness in a lizard

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Maternal nest-site choice is a behavioral phenotype with transgenerational consequences that can appear at multiple stages of offspring ontogeny. In many reptiles, the microenvironment surrounding eggs (e.g., moisture) can affect multiple aspects of offspring fitness across several life stages (e.g., embryo survival, phenotypic development, and posthatching survival). Thus, natural selection should favor maternal nesting behaviors that positively affect both embryonic and postembryonic ontogenetic trajectories. We tested this hypothesis in a 2-part laboratory experiment using the brown anole lizard (*Anolis sagrei*). In the first experiment, gravid lizards were given a choice of nesting substrates containing 5 levels of moisture content. By incubating eggs at the same 5 moisture levels, our second experiment tested if maternal choice of nest substrate facilitates embryonic development and enhances offspring quality and viability. Females strongly preferred nesting substrates with high moisture content, and these conditions yielded high hatching success, large offspring size, and overall increased offspring survival. These results suggest that selection has adaptively matched maternal nesting behaviors, embryonic development, and posthatching phenotypes in ways that enhance both offspring and parental fitness. In addition, our results highlight the importance of incorporating multiple life-history stages when assessing the fitness consequences of transgenerational effects. *Key words:* *Anolis sagrei*, brown anole, incubation moisture, maternal effects, nest-site choice, ontogenetic trajectory, phenotypic plasticity. [*Behav Ecol*]

INTRODUCTION

Evolutionary theory predicts that differential selection will favor phenotypes that enhance fitness (Darwin 1859). Although this prediction suggests that phenotypes of high fitness will occur in relatively high frequencies, the persistence of low-fitness phenotypes is still observed within populations. This persistence of less-fit phenotypes can be explained by multiple factors (e.g., constraints, plasticity, unstable environments, and mendelian inheritance) and serves as an important source of phenotypic variation on which natural selection operates (Falconer and Mackay 1996; West-Eberhard 2003). One explanation for why seemingly deleterious phenotypes persist involves variation in the timing of when fitness returns to a given phenotype are actually manifested. For example, the fitness return of a transgenerational parental effect can occur at multiple life-history stages and at different times during offspring ontogeny. Thus, an effect that has a seemingly negative impact on parental fitness may actually enhance fitness at some later point in life (Plaistow et al. 2007; Marshall 2008). These types of delayed benefits present a challenge for studies of the adaptive significance of parental effects (Marshall and Uller 2007).

The behavioral choice of oviposition site provides an excellent example of a maternal effect whose fitness consequences may be realized at 1 or more stages of offspring ontogeny. In many oviparous organisms, the developmental environment

chosen by mothers has major consequences on both embryonic and postembryonic ontogenetic trajectories (Fox et al. 1997). For example, environmental conditions of a nest site not only impact embryo survival but also the developmental trajectory of fitness-relevant phenotypes and growth in ways that can influence posthatching survival and reproductive success (Hunter et al. 2001; Jensen et al. 2009). Additionally, in some cases, conditions that maximize 1 phenotypic attribute of the offspring may not always be optimal for other phenotypes (Shine and Harlow 1996; DuRant et al. 2010). This conflict poses a major challenge to nesting females because they need to balance between sites that might optimize different aspects of their offspring's phenotype. Ideally, selection should optimize maternal nest-site choice behaviors so that parental and offspring fitness are enhanced across multiple stages of embryonic and postembryonic ontogeny (Refsnider and Janzen 2010). Thus, researchers must identify long-term effects of different developmental environments to gain insights into the adaptive value of this critical maternal effect.

Oviparous reptiles make excellent models for examining how maternal nest-site choice can impact multiple aspects of offspring fitness during embryonic and postembryonic stages. Because reptilian eggs and embryos are highly sensitive to environmental conditions and most species do not exhibit postoviposition parental care, researchers can directly evaluate the impact of maternally chosen nest conditions on ontogenetic trajectories. In nearly all species examined, thermal and hydric conditions affect hatchling phenotypes in ways that can impact fitness (Elphick and Shine 1998; Brown and Shine 2004). Moisture and temperature both significantly affect embryo development and hatching success in reptiles (Madsen and Shine 1999; Hokit and Branch 2004;

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Marco et al. 2004; Chen et al. 2010; Warner et al. 2010). These characteristics of the egg microenvironment also impact fitness-related phenotypes such as mass, length, and thermoregulatory behavior of hatchlings (Shine and Harlow 1996; Shine et al. 1997; Brown and Shine 2006; Goodman and Walguarnery 2007). Although several studies have shown that females typically choose nest sites with conditions that enhance egg hatching success (Warner and Andrews 2002a; Socci et al. 2005; Pike et al. 2010), longitudinal studies that quantify the effects of maternally chosen nest sites on posthatching ontogeny are needed to fully understand the fitness consequences of maternal nest-site choice (e.g., Huang and Pike 2011).

Considering these environmental effects on offspring phenotypes, selection should favor maternal sensory capabilities for seeking out suitable nest microhabitats for developing offspring (Warner and Andrews 2002a; Brown and Shine 2004; Socci et al. 2005; Pike et al. 2010). Our primary objective was to experimentally evaluate if lizards choose nest sites with moisture conditions that facilitate embryonic survival and phenotypic development, as well as posthatching growth and survival. We addressed these issues in a laboratory experiment using the brown anole lizard (*Anolis sagrei*). This species is well suited for addressing these issues because these lizards produce a single egg about every 7 days (Andrews and Rand 1974) and readily reproduce under captive conditions. Embryonic development of *Anolis* is sensitive to hydric conditions, which can influence several phenotypes of the offspring (Andrew and Sexton 1981; Warner et al. 2012). Our 2-part experimental design (first evaluating maternal nest-site choice, and then quantifying fitness consequences of those choices) enabled us to test the hypothesis that females would choose nest sites that maximize hatching success, as well as ontogenetic trajectories of offspring (as measured by body size, growth, and survival). Additionally, because related species often choose nest sites associated with vegetation (e.g., small plant, leaf litter; Andrews 1982; Socci et al. 2005), we incorporated an additional factor into our experiment to test if females preferentially choose oviposition sites with plants. Because plants can provide an indirect visual cue of substrate moisture availability, microhabitats containing plants might also influence maternal nest-site choice regardless of the actual moisture content of the nest substrate. Although adaptive nest-site choice is well documented in oviparous vertebrates, to our knowledge, this is the first manipulative approach to evaluate the consequences of nest-site choice across multiple life stages of offspring.

MATERIALS AND METHODS

Lizard husbandry

All lizards ($n = 80$) used in this study were wild-caught adults from Ormond Beach, Florida. Forty of these lizards (10 males and 30 females) were collected in October 2008 and maintained in a captive colony at Iowa State University before being transported to Thomas Kelly High School (Chicago, Illinois) in September 2010. An additional 40 lizards (10 males and 30 females) were wild-caught and transported directly to Thomas Kelly High School in September 2010. Adult lizards were housed among 20 cages (i.e., glass aquaria measuring 50.9 cm deep \times 31.5 cm tall \times 25.9 cm wide) covered with metal mesh tops. Each cage contained 1 male and 3 females, and lizard care generally followed the protocol of Sanger et al. (2008). Each cage contained 2 (50 cm) bamboo perches, and 10 plastic flower pots (10.4 cm long \times 10.4 cm wide \times 9.5 cm deep) filled with soil (Organic All-Purpose Potting Soil consisting of peat, perlite, and organic humus,

Peatland Garden Products LLC, Indiana) for use as nesting substrate. Gravid *Anolis*, in prior laboratory studies, readily bury their eggs in soil substrate that is provided in this study (Socci et al. 2005; Sanger et al. 2008). All cages were illuminated with ReptiSun UVB light bulbs (Zoo Med Laboratories Inc., California) set on a 13.5:10.5 h light:dark cycle. Heat was provided with 75W incandescent bulbs on a 7-h daily heat cycle. Lizards were fed live crickets dusted with calcium and reptile vitamins twice per week and misted with water 2 times per day on weekdays. Misting was evenly distributed over each cage. On weekends, a petri dish filled with water was placed in each cage. This experiment was conducted in a classroom at Thomas Kelly High School, and students were responsible for most of the animal care and data collection.

Experiment I: nest-site choice

Each cage provided females with 10 choices of oviposition sites that varied in the level of substrate moisture and the presence of a plant. About 82% of the bottom of each cage was covered with plant pots (i.e., nest-boxes) containing potting soil with different moisture contents. Each cage contained pots with soil moisture contents of 0%, 12.5%, 25%, 50%, and 75% (within a range likely encountered in the wild; see Discussion). All soil was initially dried to 0% moisture, and each pot with soil was measured for mass and water was added (and thoroughly mixed) as a percentage of dry mass to establish these 5 levels of substrate moisture. Each moisture level was present in 2 pots (i.e., nest-boxes) out of the total 10 per cage. A plant (*Philodendron cordatum*) was planted in 1 pot at each moisture level in each cage (5 plants per cage). Due to a limited number of live plants, some cages contained 2 artificial *P. cordatum* plants (with 3 live plants), and other cages contained 3 artificial plants (with 2 live plants), such that overall half the plants used were live. The positions of the 10 pots were randomized within each cage, and pot positions were rotated each week to account for any bias in the nesting data based on the position of a nest-box within a cage.

Once per week (from 14 September to 19 November 2010), we carefully sifted through the soil in each pot (and temporarily removed plants from pots) to search for eggs. For each egg found, we recorded the presence or absence of a plant, whether the plant was artificial or live, soil moisture, and pot number. All the soil was then carefully added back to the pot. Each pot was then measured for mass and water was added back until it was restored to its original mass to replace moisture lost through evaporation. All soil was then thoroughly mixed and any removed plants were then repotted.

Experiment II: effect of incubation moisture on posthatching viability

Although data collection for experiment I ended on 19 November 2010, we continued collecting eggs for experiment II until 20 January 2011. Each egg ($n = 129$) was immediately measured for mass (to 0.01 g), and randomly assigned to 1 of 5 soil incubation treatments that mirrored the substrate moisture levels available for nesting (0%, 12.5%, 25%, 50%, and 75%). Although egg collection took place between 0 and 7 days after oviposition, our random assignment of eggs to treatments ensured that the average age of eggs was similar among treatments. Moreover, substrate moisture does not affect water uptake by *A. sagrei* eggs during the first week after oviposition (Warner et al. 2011, 2012), suggesting that pre-treatment moisture conditions should have minimal (if any) phenotypic effects. Eggs were loosely covered in soil within plastic Petri dishes, and lids were tightly affixed with adhesive tape. Eggs were incubated in a darkened incubator at a

constant temperature of 27 °C. The soil in each incubation treatment was replaced weekly with freshly mixed soil at the designated moisture level to account for evaporative moisture loss within the incubator. Eggs were checked for hatching twice daily on weekdays. All hatchlings were sexed, measured for mass, snout-vent length (SVL), and tail length (TL), and toe clipped for individual identification.

Hatchlings ($n = 41$) were reared in plastic enclosures (36.8 cm deep \times 22.2 cm wide \times 24.8 cm high) with no more than 3 individuals per enclosure. Lighting and heat followed the same cycles as for the adult lizards (13.5 h light/10.5 h dark and 7 h, respectively). Potting soil was used as substrate, and artificial *Philodendron* plants provided perches and shelters. Each enclosure contained an open Petri dish (lined with gravel) filled with water. Enclosures were misted with water twice daily. Hatchlings were fed small crickets twice weekly. Hatchlings were measured (mass, SVL, and TL) every 3 weeks to track growth up to 12 weeks. Because hatchlings were observed daily, dead individuals were identified and immediately removed from enclosures when found, and the date of mortality was recorded.

Statistical analysis

Nest-site choice in relation to substrate moisture and the presence of a plant was quantified with chi-square tests. Because 3 females were housed per cage, we could not assign maternal identity to each egg, and therefore these analyses treated each egg independently. However, for a complementary analysis that accounted for “cage effects,” we calculated the percentage of eggs laid in each substrate type (i.e., moisture and plant) for each cage, and used these percentages as our unit of analysis in a mixed-model analysis of variance (ANOVA). An additional chi-square test was used to evaluate maternal choice of a live versus artificial plant for oviposition site.

The effect of incubation moisture on egg survival was evaluated with a logistic regression model that included incubation moisture, egg mass, Julian date of oviposition, and cage as independent variables, and hatching success (yes vs. no) as a dependent variable. A mixed-model ANOVA was used to evaluate the effect of incubation moisture on incubation duration (days spent in incubation treatment to hatching). Mixed-model analysis of covariance (ANCOVA) was used to evaluate the effect of incubation moisture on hatchling SVL, TL, body mass, and body condition. Egg mass was used as a covariate for analyses of SVL and body mass, and SVL was a covariate in analyses of TL and body condition (i.e., mass relative to SVL).

Mixed-model repeated-measures ANOVA was used to evaluate the effect of incubation moisture on change in hatchling body size (i.e., growth). This analysis was performed twice, first using SVL, and then with body mass as the dependent variables. In a second analysis of hatchling growth, we calculated the change in SVL divided by the number of days between measurements (21 days) for the first 3 weeks, 3–6 weeks, and 6–9 weeks after hatching. Treatment effects on growth during each of these periods were evaluated with mixed-model ANCOVAs using SVL at the onset of each growth period as a covariate. Hatchling survival was evaluated with generalized linear mixed models at 3, 6, 9, and 12 weeks after hatching; separate models at each time period included incubation moisture, Julian day of hatching, and hatchling mass as independent variables. In a final analysis of overall offspring viability, we used generalized linear mixed models (similar to that described for hatchling survival) to evaluate the overall effect of incubation moisture on survival up to 12 weeks posthatching. This analysis combined survival data during both embryonic and postembryonic stages (rather than

just posthatching survival) to gain a more comprehensive picture of moisture effects on overall survival.

Prior to all parametric analyses, data were checked for normality and log-transformed when necessary. Data from wild-caught and laboratory-reared lizards were pooled for all analyses because preliminary results showed minimal differences between these 2 groups (differences in egg mass, $P = 0.195$; hatchling morphology, all P values > 0.165 ; hatchling growth, all P values > 0.241 ; hatchling survival, $P = 0.282$). For all ANCOVAs, interactions between moisture treatment and covariates were never significant (i.e., homogeneity of slopes tests) and these interaction terms were removed from final models (Engqvist 2005). For all mixed-model analyses, cage number was designated as a random effect to control for differences among cages. Hatchling sex was not considered as a factor in any analysis because males and females did not differ in any trait that we measured (all P values > 0.112). All analyses were performed with SAS software (version 9.2, SAS Institute 1997).

RESULTS

Nest-site choice

Microhabitat data (moisture and plant) were collected for 61 nests. Females strongly preferred relatively moist nesting substrates ($\chi^2_4 = 78.8$, $P < 0.001$; Figure 1), with 91.9% of the eggs oviposited in the substrates with the highest moisture levels (75% and 50% moisture). For analyses restricted to the 2 highest moisture substrates, a preference for moisture was still evident, with 36 eggs in substrates with 75% moisture and 21 eggs in substrates with 50% moisture content ($\chi^2_1 = 3.9$, $P = 0.047$). Only 8.1% of the eggs were oviposited in substrates with 25% moisture or lower, and no eggs were recorded at the lowest available moisture level (0%). These results were corroborated by analyses that accounted for cage effects ($F_{4,68} = 17.7$, $P < 0.001$). Females nested randomly with respect to the presence of a plant ($\chi^2_1 = 0.1$, $P = 0.811$), and did not show any preference for live versus artificial plants ($\chi^2_1 = 0.1$, $P = 0.732$).

Effect of incubation moisture on development and hatchling quality

Overall, hatching success was 32.6% (out of 129 eggs), and was strongly affected by the moisture level of the incubation substrate (Table 1). Eggs from relatively moist incubation treatments had substantially greater survival than those from relatively dry treatments (Figure 2a). Accordingly, all

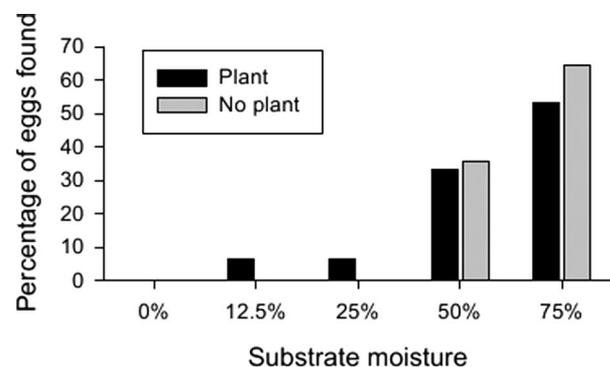


Figure 1 Nest-site choice in relation to substrate moisture content and the presence of a plant.

Table 1

Effect of egg incubation moisture on development, hatchling morphology, growth, and survival

Variable	Covariate(s)	Incubation moisture effect
Development		
Egg survival	—	$\chi^2 = 16.0$, $P = \mathbf{0.003}$
Time until death (days)	—	$F_{4,65} = 4.9$, $P = \mathbf{0.002}$
Incubation duration (days)	—	$F_{2,28} = 0.7$, $P = 0.523$
Hatchling morphology		
SVL (mm)	Egg mass	$F_{2,27} = 4.1$, $P = \mathbf{0.029}$
Tail length (mm)	SVL	$F_{2,27} = 0.4$, $P = 0.691$
Body mass (g)	Egg mass	$F_{2,27} = 7.0$, $P = \mathbf{0.004}$
Body condition (mass, g)	SVL	$F_{2,27} = 4.2$, $P = \mathbf{0.026}$
Growth rate (Δ SVL/days)		
Through first 3 weeks	SVL at hatching	$F_{2,21} = 0.6$, $P = 0.557$
Through second 3 weeks	SVL at 3 weeks	$F_{2,11} = 2.2$, $P = 0.156$
Through third 3 weeks	SVL at 6 weeks	$F_{2,5} = 1.5$, $P = 0.316$
Hatchling survival		
At 3 weeks	Mass and date at hatching	$F_{2,36} = 0.3$, $P = 0.722$
At 6 weeks	Mass and date at hatching	$F_{2,24} = 2.2$, $P = 0.075$
At 9 weeks	Mass and date at hatching	$F_{2,20} = 1.1$, $P = 0.339$
At 12 weeks	Mass and date at hatching	$F_{2,19} = 1.2$, $P = 0.338$
Overall survival (eggs + hatchlings)		
At 3 weeks	—	$F_{2,85} = 11.2$, $P < \mathbf{0.001}$
At 6 weeks	—	$F_{2,83} = 11.5$, $P < \mathbf{0.001}$
At 9 weeks	—	$F_{2,79} = 7.3$, $P = \mathbf{0.001}$
At 12 weeks	—	$F_{2,78} = 5.3$, $P = \mathbf{0.007}$

P values in bold face are statistically significant.

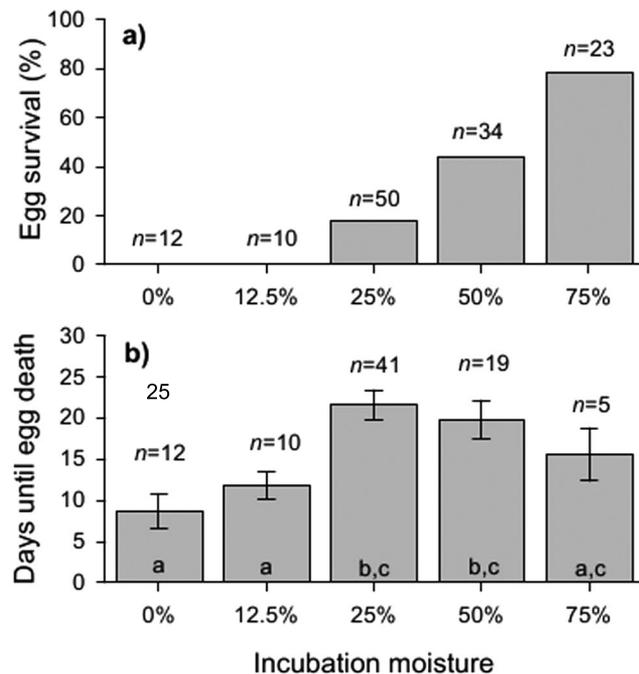


Figure 2

Effect of incubation moisture on (a) hatching success of eggs, and (b) the number of days until signs of egg mortality became apparent; least-square means are reported, and error bars represent 1 SE. Sample sizes for each treatment are indicated on the figure; after it became obvious that eggs quickly desiccate and die under dry conditions (0% and 12.5% moisture), we ceased allocating eggs to these 2 treatments in order to increase hatchling sample sizes in the other treatments (25%, 50%, and 75% moisture). Treatments with the same letter are not significantly different from each other.

eggs from dry incubation conditions (0% and 12.5% moisture) died, and these eggs showed signs of mortality (shriveled appearance due to negative water balance) much sooner

than eggs incubating under relatively moist conditions (Figure 2b). For eggs that survived to hatching, incubation duration was not affected by substrate moisture (overall mean \pm SD = 32.0 \pm 2.7 days; Table 1). Although variation in egg survival was not explained by egg mass ($\chi^2_1 = 0.1$, $P = 0.782$) or oviposition date ($\chi^2_1 = 0.5$, $P = 0.465$), these 2 factors influenced variation in incubation duration; incubation duration was negatively related to egg mass ($r^2 = -0.12$, $P = 0.025$) and positively related to oviposition date ($r^2 = 0.12$, $P = 0.027$). Variation in egg survival and incubation duration was not influenced by maternal cage (P values > 0.9).

A total of 41 eggs successfully hatched. Hatchlings from the highest moisture treatment (75% moisture) were significantly larger in SVL and mass than hatchlings from the other treatments, but those from the 25% and 50% treatments did not differ from each other (Table 1, Figure 3). Hatchlings from the 25% moisture treatment had a relatively low average body condition, but those from the 50% and 75% treatments did not differ from each other (Table 1, Figure 3). Incubation moisture had no effect on hatchling TL (Table 1). Hatchling growth rate was not influenced by incubation moisture during the first 9 weeks after hatching (Table 1), and sample sizes were not large enough (due to mortality) to confidently evaluate treatment effects on growth beyond this time period. Nevertheless, because hatchlings from the 75% moisture treatment were relatively large at hatching, these individuals maintained their size advantage for several weeks after hatching (albeit, this size difference was no longer significant at 6 weeks after hatching; Figure 4a). These patterns were similar for analyses of growth based on SVL or body mass.

Hatchling survival was relatively high during the first 3 weeks after hatching (87.8%), but decreased to 32.4% by 12 weeks of age. Although treatment differences in survival rates were not statistically significant (Table 1), hatchlings that developed under relatively moist incubation conditions had relatively high survival (Figure 4b). Despite relatively high egg survival from the 50% incubation treatment, this incubation condition resulted in the lowest survival rates for hatchlings; by 12 weeks, survival in this treatment was 5–6 times lower

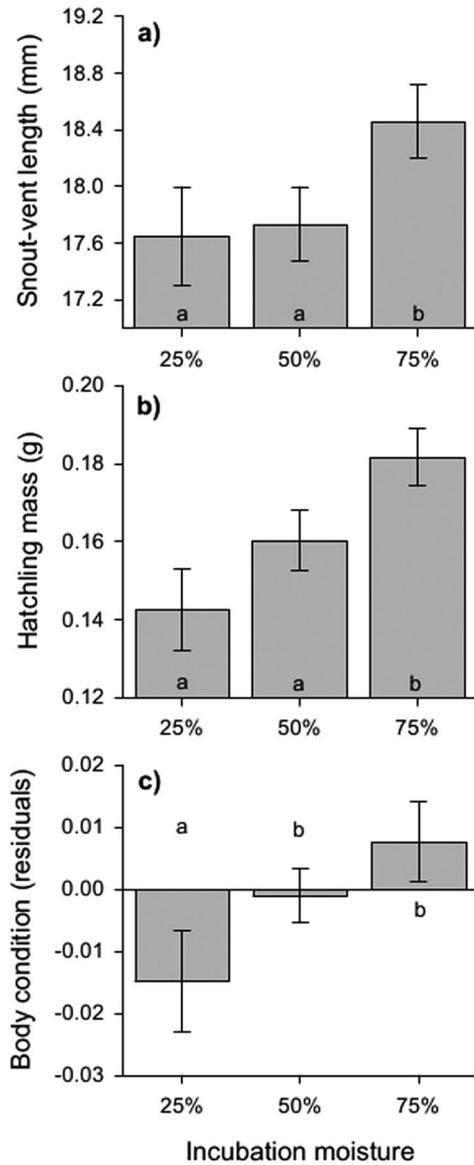


Figure 3
Effect of egg incubation moisture on hatchling (a) SVL, (b) body mass, and (c) body condition. Body condition is represented as residual scores from the regression of body mass on SVL. Least-square means are reported, and error bars represent 1 SE. Treatments with the same letter are not significantly different from each other.

than that in the 25% and 75% moisture treatments. Egg mass and oviposition date did not explain variation in hatchling survival at any time period (all P values > 0.05). Incubation at 75% moisture resulted in substantially higher overall offspring viability (eggs and hatchlings combined) than incubation at 25% or 50% moisture (Table 1, Figure 4c).

DISCUSSION

Because variation in egg survival, offspring phenotypic development, and posthatching survival can be influenced by developmental environments, adaptive nest-site choice can become apparent via fitness consequences across multiple life-history stages of the offspring (Refsnider and Janzen 2010). The main objective of our study was to test

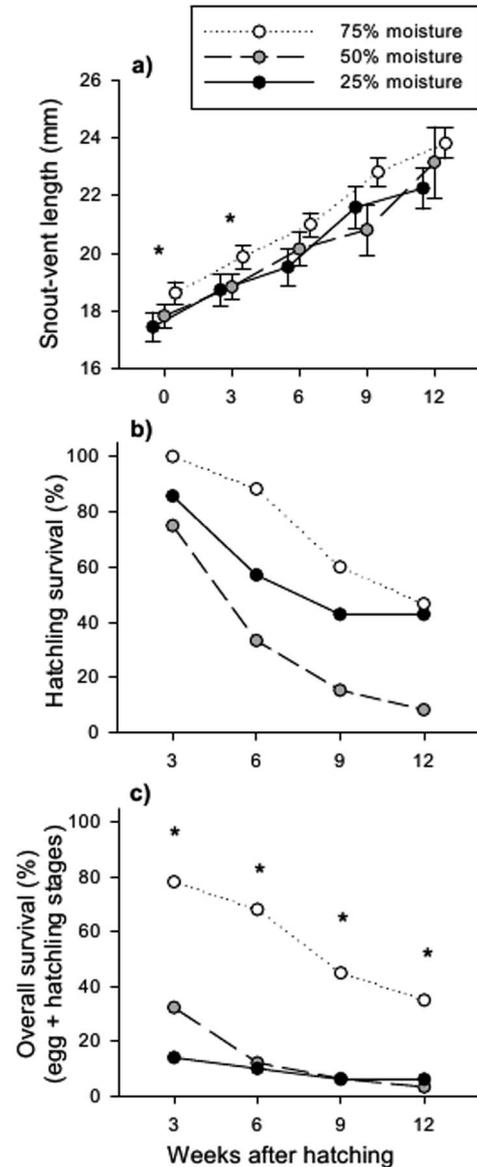


Figure 4
The effect of egg incubation moisture on (a) change in SVL, and (b,c) hatchling survival through 12 weeks after hatching. For growth results (a), sample sizes for the 25% and 50% moisture treatments were relatively low (due to mortality) at 9 and 12 weeks of age, and may explain the “apparent shifts” in growth among the incubation moisture treatments at these time periods. For graph b, percentage of survival is calculated using only individuals from eggs that successfully hatched. Graph c represents overall survival that combines embryonic and postembryonic stages. Least-square means are reported and error bars represent 1 SE. Asterisks denote statistical significance ($P < 0.05$).

this assertion and determine if females demonstrate adaptive nesting behaviors that not only enhance embryo survival but also increase posthatching offspring quality and survival. Specifically, our experiment was designed to determine if female lizards were sensitive to subtle moisture cues when selecting nest sites, and if maternally chosen moisture levels influence multiple aspects of offspring fitness. Overall, our data strongly support our predictions, as females selected nest sites that enhance hatchling success, and these same conditions also had positive effects on offspring quality during early life stages (in terms of size, growth, and survival). Thus,

our findings provide strong experimental evidence for adaptive nest-site choice in reptiles.

Nest-site choice

Female *A. sagrei* clearly preferred relatively moist nest sites. This finding generally corroborates other studies of reptiles that have explored nesting behavior in relation to substrate moisture availability (Plummer and Snell 1988; Socci et al. 2005; Warner and Andrews 2002a). Although substrate moisture content was the primary cue for nest-site choice in our study, indirect cues of moisture availability (i.e., presence of a plant) did not impact maternal nesting behavior. These results suggest that female *A. sagrei* have well-developed sensory abilities and are capable of detecting subtle variation in substrate moisture levels in relatively small areas without need to evaluate indirect cues. Although lizards in our experiment demonstrated the ability to perceive differences in the moisture gradient available (0%, 12.5%, 25%, 50%, and 75%), future studies with more subtle differences in moisture availability would be useful in showing the ability of females to detect moisture at a finer resolution. Additionally, individual females may vary in the strength of their preference for moisture in nest sites because of family specific variation in egg desiccation resistance or in embryo developmental responses to moisture. Although our study demonstrates that females choose moisture levels that optimize growth and survival of offspring, our study was not designed to evaluate individual variation in nest-site choice and family specific effects on offspring growth and survival. Future studies that match individual eggs to specific females would be useful in addressing this issue.

The ability to detect such moisture variation at potential nest sites is likely to be important in the wild. Although we do not know specific nest conditions chosen by female *A. sagrei* in the field, the moisture levels used in our study likely encompass those available. Indeed, *A. sagrei* inhabits both urban and nonurban environments that likely contain very dry (e.g., due to drought) and very wet (e.g., due to artificial sprinkling systems) substrates that occur in close proximity (Reedy and Warner, personal observation). Additionally, previous work has shown a similar wide range of substrate moistures within potential nest sites of another lizard species that occurs sympatrically with *A. sagrei* (Robbins and Warner 2010). Given that *A. sagrei* inhabits extremely dry areas (in its native and introduced range), the ability to detect subtle moisture variation could be vital for population recruitment and persistence.

Because lizards preferred to nest in the moistest treatment available, would our results change if the range of moisture conditions extended beyond those used in this experiment? Indeed, our range of moisture conditions did not enable us to detect if even greater moisture contents would discourage nest-site choice. Although this was a limitation of our study, soils with moisture contents much beyond 75% would have been saturated (or nearly so) with water, and we expect that females would avoid nesting in substrates with such high moisture contents. Although *A. sagrei* eggs can withstand complete submersion for short periods (Losos et al. 2003), saturated conditions have negative impacts on embryonic development because they can facilitate fungal growth (Tracy 1980) and disrupt proper O₂ exchange between the eggs and environment (Ackerman et al. 1985; Kennett et al. 1993; Tucker et al. 1997).

Effect of incubation moisture on development and hatchling quality

The moistest incubation condition (75% moisture) yielded high hatching success and produced relatively large

hatchlings with enhanced body condition (Figure 3). This pattern is in agreement with a large body of research showing that relatively moist incubation conditions facilitate yolk metabolism during embryogenesis, resulting in larger offspring compared with eggs from dry incubation substrates (Packard and Packard 1988; Warner et al. 2012). Because large body size often enhances performance and survival of hatchling reptiles (Ferguson and Fox 1984; Sinervo et al. 1992; Janzen 1993), the effect of incubation moisture can have considerable fitness consequences. Indeed, our data show a 12% and 22% difference in mass between hatchlings incubated at the 75% moisture treatment versus hatchlings from the 50% and 25% moisture treatments, respectively. This variation in body size due to incubation moisture exceeds that previously reported for *A. sagrei* (albeit, incubation moisture conditions were less extreme in previous work; Warner et al. 2012) and resultant effects on survival were evident. Given the possibility of genetic variation in how embryos respond to moisture conditions, future research should address this genetic by environment interaction to further assess how mothers might adaptively choose nest sites based on reaction norms of their own offspring (Shine et al. 1997).

The effect of incubation moisture on growth rate and survival of hatchlings was not statistically significant, but individuals from the 75% moisture treatment remained relatively large and had substantially high survival for several weeks posthatching. How this body size advantage (due to relatively moist incubation conditions) influenced fitness is unknown, but plausibly could enhance performance (e.g., locomotion and bite force) in ways that could minimize predation (Warner and Andrews 2002b) or increase foraging efficiency (Verwajen et al. 2002). In this study, the possibility of predation was eliminated, but variation in growth and survival could have been influenced by competition for food (Ferguson and Fox 1984; Smith and Ballinger 1994; Manteuffel and Eiblmaier 2010). Hatchlings from the 75% moisture treatment had substantially high survival during the first 9 weeks posthatching, suggesting that moist incubation environments may enhance survival during critical early life stages. In contrast to expectations, however, hatchlings from the 50% moisture treatment had lower survival than those from the 25% treatment. Although this result warrants further study, by combining embryonic and postembryonic survival, we show that overall offspring fitness does not differ between the 25% and 50% treatments. Early life stages of many reptiles are sensitive to predation and competition (Head et al. 2002; e.g., Laurie and Brown 1990; Manteuffel and Eiblmaier 2010; Tomillo et al. 2010; but see Pike et al. 2008), thus any factor that enhances variation in survival or fitness-related phenotypes at these early stages (e.g., maternal nest-site choice of incubation conditions) would be an important target of natural selection.

CONCLUSIONS

Maternal nest-site choice is a behavioral phenotype with transgenerational consequences that can appear at multiple life stages. Therefore, the fitness returns of nest-site choice may not be fully manifested until relatively late in offspring ontogeny. Thus, studies that focus on single estimates of fitness at only 1 life stage can misdirect assessments of the adaptive significance of nest-site choice. Our longitudinal experimental study addressed this issue by showing that moisture conditions chosen by females not only facilitate successful embryonic development but also positively affect offspring phenotypes and survival. For example, most females in our study chose the moistest conditions available for

nesting, which had clear benefits to overall offspring viability (Figure 4c).

Because overall fitness is a result of several events accumulated across multiple life-history stages (e.g., develop properly to hatch, grow to reach maturity, survive to reproduce), adaptive evolution of nesting behavior may have occurred in a hierarchical fashion. For example, nesting behaviors first may have been shaped by limitations of egg/embryo physiology that affect embryonic survival, but secondary selection may have shaped maternal behaviors that positively affect posthatching ontogeny and offspring survival. Because effects of egg incubation environments can persist to adulthood (Gutzke and Crews 1988; Warner and Shine 2008), future studies should incorporate the impacts on adult reproductive success to fully understand the adaptive value of nest-site choice. Although we did not evaluate adult stages, our study provides experimental evidence that selection has shaped a match among maternal reproductive behaviors, embryonic development, and posthatching phenotypes and survival in ways that enhance parental fitness.

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