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THE INFLUENCE OF NATURAL INCUBATION ENVIRONMENTS ON THE PHENOTYPIC TRAITS OF HATCHLING LIZARDS

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Abstract. Laboratory studies have shown that incubation environments can affect morphological and behavioral phenotypes of hatchling lizards, but the relevance of this result to natural populations remains unclear. We monitored thermal regimes during the incubation period in 19 natural nests of scincid lizards (*Bassiana duperreyi*) in montane southeastern Australia, and experimentally translocated eggs among nests to remove the confounding of ''nest of origin'' (including genetic) factors with incubation conditions. We removed the eggs from the field shortly before hatching, and assessed the hatchlings' phenotypes (body size, shape, locomotor performance). Most of the effects seen after laboratory incubation were also seen after incubation in natural nests. Hatchling phenotypes were affected by incubation conditions as well as by ''nest of origin'' factors and an interaction between the two. Both the mean and the variance of incubation temperatures affected hatchling phenotypes, with male and female hatchlings differing in their norms of reaction. We found no evidence that a female's choice of nest site depends on the specific norms of reaction of her own offspring. Overall, incubation temperatures induced approximately half as much variance in hatchling phenotypes as did ''nest-of-origin'' effects. We conclude that incubation-induced phenotypic plasticity in hatchling reptiles may be important in the natural environment, as well as in the laboratory.

Key words: Australian Scincidae; Bassiana duperreyi; *Brindabella Ranges, southeastern Australia; embryogenesis, thermal conditions; lizard hatchlings; phenotypic variance, reptiles; temperature fluctuations and hatchling traits; thermal biology.*

INTRODUCTION

The role of direct environmental influences on phenotypic traits has attracted increasing scientific attention in recent years, and reptiles have proved to be excellent model systems for research in this field. Research on phenotypic plasticity in these animals was stimulated by the discovery of temperature-dependent sex determination (e.g., Bull 1980), leading investigators to ask if incubation regimes (especially, thermal and hydric environments during incubation) affected aspects of the animal's phenotype other than sex determination. Many such effects have now been documented, using a diversity of reptile taxa (turtles, crocodilians, snakes, lizards) and examining a number of different organismal traits involving morphology, locomotor performance, and general behavior (see reviews in Rhen and Lang 1995, Shine and Harlow 1996, Roosenburg 1996). This work has led to suggestions that incubation-induced phenotypic plasticity may play an important role in many biological processes, including ecological phenomena (e.g., nest-site selection, micro- and macrogeographic variation in life histories: Gutzke and Packard 1987, Viets et al. 1993, Resetarits 1996, Roosenburg 1996, Shine and Harlow 1996) as well as evolutionary phenomena (e.g., shifts in reproductive mode, maternal behavior, mode of sex determination: Rhen and Lang 1995, Shine 1995, Shine et al. 1995, Tousignant and Crews 1995, Qualls and Shine 1997).

This literature has shown an increasing methodological sophistication. Most early experiments relied on constant-temperature incubation, but more recent work has attempted to measure and then simulate conditions experienced in natural nests (e.g., Shine and Harlow 1996). Inevitably, this is difficult to do: natural nests show enormous spatial and temporal variation in traits such as the means and variances of temperature and soil moisture levels (Packard et al. 1977, Packard and Packard 1988, Palmer-Allen et al. 1991). Thermal variances as well as mean temperatures can affect hatchling phenotypes (Shine and Harlow 1996). Even when experiments simulate appropriate regimes with respect to one variable (e.g., temperature), they may introduce artifacts with another (e.g., moisture). For example, it is almost impossible to ensure that eggs incubated at different temperatures are maintained at identical water potentials (Packard 1991, Shine 1995). Although similar functional dependencies among physical conditions may also occur in natural nests, we do not know if this is the case.

In summary, laboratory experiments show that in-

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cubation conditions can influence the phenotypes of hatchling reptiles, but it is difficult to extrapolate this result to the field. For example, natural nests within a population may not vary enough in incubation conditions to engender significant phenotypic variance in hatchlings. Even if nests vary in such ways, successful hatching may only occur from a subset of nests—potentially, those with a restricted set of incubation conditions. Alternatively, even if factors such as temperature and soil moisture levels vary among natural nests to a degree that would influence hatchling phenotypes if each factor acted alone, the overall impact of these factors may be reduced by the patterns of covariation of temperature and moisture in natural nests.

The only way to overcome laboratory artifacts such as these is to study eggs in natural nests. This technique has been adopted in studies of temperature-dependent sex determination, and has demonstrated that clutches from natural nests display sex-ratio biases consistent with those predicted from laboratory studies (Bull 1980, Janzen and Paukstis 1991, Roosenburg 1996). However, effects of incubation conditions on other aspects of the hatchling phenotype (morphology, behavior, locomotor performance) are difficult to separate from maternal (including genetic) effects on these attributes, if all that is available is information on the phenotypes of naturally incubated hatchlings. The central problem is that in nature, hatchlings emerging from a single nest will be similar in their genetic constitution as well as in the physical conditions that they have experienced during incubation. Thus, any consistent among-nest differences in phenotypic traits of hatchlings may be due to genes (or more generally, to ''nest-of-origin'' effects) rather than to incubation regimes. In order to separate out these confounding factors, we moved eggs from one nest to another. By randomizing the genetic constitution of the hatchlings emerging from each nest, any consistent differences in the phenotypes of hatchlings emerging from different nests must be due to the nest environment rather than to maternal factors (Packard et al. 1993, Cagle et al. 1993).

This experimental design also allowed us to quantify the proportion of phenotypic variance in each trait attributable to incubation effects vs. maternal (including genetic) factors, and to examine the validity of several conclusions from laboratory experiments—for example, the notions that hatchling phenotypes are influenced by thermal variance as well as by the mean incubation temperature (Shine and Harlow 1996), and that the sexes respond differently to the conditions they experience during incubation (Shine et al. 1995). Lastly, we were able to test the idea that among-clutch differences in the reaction norms of embryos influence maternal nest-site selection, so that mothers deposit their eggs in nests best-suited to their own embryos (Shine and Harlow 1996).

Materials and Methods

Our study species, *Bassiana duperreyi*, is a small (to 80 mm snout–vent length), oviparous, scincid lizard widely distributed in cool-climate habitats in southeastern Australia (Cogger 1992). Females produce a single clutch of three to nine eggs once per year, in early summer (Pengilley 1972, Shine 1983, 1995). The eggs are laid under rocks and logs in areas exposed to full sun for much of the day, and females appear to select oviposition sites based upon the mean and variance of temperatures that their eggs are likely to experience during incubation (Shine and Harlow 1996). Communal oviposition is common (Pengilley 1972, Shine 1983, 1995, Shine and Harlow 1996). Our study sites were in the Brindabella Ranges, 40 km west of Canberra in the Australian Capital Territory. We worked at two open areas ~9 km apart (Picadilly Circus: 1246 m elevation, 148°50′ E, 35°21′ S; Coree Flats, 1050 m, 148°48′ E, 35°17′ S). Female *B. duperreyi* congregate in these places in early summer to lay their eggs, apparently because of the greater exposure to direct sunlight compared to the surrounding forest (swathes of trees have been felled in both areas, to prevent interference with overhead powerlines).

Oviposition is highly synchronous among the female lizards in this high-elevation environment, although the exact timing shifts from year to year depending on weather conditions (Pengilley 1972). We visited these areas on 15–17 December 1995, midway through the oviposition period, and searched for natural nests by turning over all suitable rocks and logs. We removed all eggs and placed groups of four to six eggs in short (8-cm) lengths of nylon stocking, tied at each end. The open weave of the stockings allowed ample access to air and to moisture from the surrounding soil. All the packets were labelled with the identity of their nest of origin, and allocated randomly between two treatments. Even in communal nests, it was usually possible to recognize individual clutches because of the consistent sizes, shapes, and locations of groups of eggs. Nonetheless, our analyses rely upon ''nest-of-origin'' effects rather than ''clutch'' effects, because of ambiguity about clutch affinities in some cases. Half of the eggs from each nest were returned to the original nest, and the other half were transferred among other nests. The destination nest was decided randomly, within the constraint that the number of translocated eggs placed in a nest was equal to the number that had been removed. This constraint enabled us to fit the eggs into the cavity left when other eggs had been removed, without requiring additional excavation (which may have changed the thermal or hydric regimes experienced by the eggs). It also meant that the overall distribution of incubation environments was the same for the ''replaced'' and ''translocated'' eggs, and that both groups retained the original distribution of eggs among nest environments.

In the interval (usually <4 h) between their removal from one nest and their replacement into another or the same nest, the eggs were kept (in their stocking bags) in large containers of moist vermiculite at shaded air temperatures. We carefully recorded the position of the rocks or logs covering each clutch, and attempted to replace the cover object in its original position. Probes attached to thermal data loggers (Hobo-temp, Onset Computer Corporation, Pocasset, Massachusetts, USA) were placed in the middle of the egg mass in each nest.

We returned to the study sites on 6 February 1996, when we calculated that the first eggs would be hatching. At this time we removed all eggs, and the accompanying data loggers, and checked the oviposition sites for eggs laid subsequent to our initial visit. The eggs (still in their nylon packages) were transferred to the University of Sydney in large containers filled with moist vermiculite. At the University, we opened all of the packages, and transferred each batch of eggs to individual 250-mL glass jars for incubation. The vermiculite in these containers (as in all other containers used to transport eggs) had a water potential of −200 kPa; evaporation was prevented by covering the open top of the jar with plastic foodwrap (see Shine and Harlow [1996] for calibration details). All eggs were incubated under the same thermal regime, set to mimic a natural nest (i.e., sinusoidal daily variation from 14° to 26°C, over ten steps, in a programmable incubator). Incubators were checked daily, and all hatchlings were weighed, measured (snout–vent length [=SVL], tail length), and then maintained (separately for each package cohort) in 22 × 13 × 7 cm containers with ad libitum water. The containers were kept in a room with a constant air temperature of 20°C, but heating strips running underneath one end of each container enabled the hatchling lizards to select body temperatures over the range 20° to 38°C for 9 h each day.

We measured locomotor ability of the hatchlings when they were 7 d of age. Running speeds were measured at 25°C, using a 1-m raceway with photocells at 25-cm intervals. Hatchlings were allowed to equilibrate to room temperature (25°C) for 30 min (see Elphick 1995), and then placed at one end of the raceway. An artist's paintbrush was used to ''chase'' the lizard, and we recorded the time it took to cover each 25-cm interval. Each lizard was raced three times, with a minimum of 15 min rest between successive runs. The lizards were then reweighed and remeasured, to assess growth during their 1st wk after hatching. Later, all of the hatchlings were sexed by manual eversion of the hemipenes, after cooling on ice until they were immobile (Harlow 1996). About half of the lizards had relatively large (0.9–1.2 × 0.4–0.5 mm) pink hemipenes, whereas the others had small (0.2–0.4 × 0.1–0.2 mm) white structures similar in shape to hemipenes. The accuracy of this technique was confirmed by dissection of 12 skinks after sexing; all of those with hemipenes ($n = 7$) lacked oviducts, whereas all of those without hemipenes ($n = 5$) had macroscopically visible oviducts. The remaining lizards were then returned to the Brindabella Ranges, and released on the study sites.

Our data on hatchling phenotypes (incubation periods, morphology, locomotor performance, growth) were analyzed in several ways, including analysis of variance (ANOVA), analysis of covariance (ANCOVA), and linear regression. For traits that were highly correlated (e.g., mass relative to length), we calculated residual scores from the general linear regression of one variable against the other and used these residual scores as dependent variables for subsequent analyses of body shape (ln-transformed mass vs. SVL), relative tail length (tail length vs. SVL), and growth rate (size at 1 wk of age regressed on size at hatching). Because of intercorrelations among variables, and problems associated with spuriously ''significant'' results from multiple tests, we used multivariate as well as univariate ANOVAs to examine the statistical significance of overall effects on hatchling phenotypes.

Results and Discussion

Number of eggs

We located 19 nests, containing a total of 372 eggs. Given a mean clutch size of 5 eggs for *Bassiana duperreyi* in this population (Shine and Harlow 1996), these eggs comprised ~74 clutches. Of the 372 eggs present at our first visit, 179 eggs were returned to their nest of origin, and the other 193 eggs (52%) were translocated to other nests. Three nests were omitted from our treatments because they contained too few eggs for experimental manipulation.

Survival of eggs

Of the 372 eggs that we located and ''packaged'' in December 1995, 88% were still viable in February 1996 when we returned to the nests. An additional 48 eggs had been laid in six of the nests, indicating that we had been successful in timing our initial trip to be midway through the nesting season, and that our manipulations had not deterred other females from using the same sites.

Incubation periods

None of the 372 eggs that we ''packaged'' in December 1995 had hatched prior to our return visit in February 1996. Hatchlings began to emerge 6 d after the eggs were placed in the incubator; the last hatchling emerged 44 d later. Thus, our hatchlings spent an average of 71% of their total incubation in natural nests (range: 54 to 90%), with the remainder in the laboratory incubator.

Nest temperatures

The thermal regimes of our 19 natural nests (as measured by data loggers over the first 7 wk of incubation, up to 6 Feb 1996) varied considerably among nests. Mean daily minimum temperatures ranged from 11.5°

to 15.3°C, and mean maxima from 20.6° to 29.7°C. Grand means ranged from 16.8° to 20.1°C. Hence, our laboratory incubation treatment provided a thermal regime similar to those experienced by eggs in natural nests.

Hatchling phenotypes

Hatchling lizards ranged from 23.0 to 29.5 mm SVL (snout–vent length), had tails of 22.5 to 36.0 mm in length, and weighed 0.17–0.35 g. The young lizards also varied considerably in locomotor performance, with maximal running speeds (over the fastest 25-cm segment) of 0.07 to 0.93 m/sec, and overall speeds (over the full 1-m racetrack) of 0.06 to 0.60 m/sec. Sex ratios varied from 28 to 71% male among eggs from the 16 different nests, with an overall average of 51% (1 SD = 9.0%; n = 162 males, 158 females). These numbers do not diverge significantly from a 50:50 ratio, and contingency-table analysis shows that that the among-nest variation in sex ratios was not statistically significant ($\chi^2 = 7.12$, 15 df, $P = 0.95$).

Do hatchling phenotypes differ between eggs from different nests?

The first prediction from the hypothesis of incubation-induced phenotypic effects is that there will be significant among-nest variation in the phenotypes of hatchling lizards. To test this prediction, we used nest identification number as the factor in a one-factor MANOVA on the data for eggs that had been returned to their original nests for incubation. These represent the ''natural'' condition for this population. Our test revealed significant differences among nests in the phenotypes of hatchling lizards within this group (Hotelling-Lawley trace = 8.48, $F_{240, 1847} = 4.35$, $P < 0.0001$). More detailed examination using a series of one-factor ANOVAs showed that this significant result was due to strong differences among nests in traits such as incubation period ($F_{15, 140} = 37.53$, $P < 0.0001$), and hatchling morphology (mass: $F_{15, 140} = 5.62$, $P < 0.0001$; SVL: $F_{15, 140} = 4.78$, $P < 0.0001$; tail length: $F_{15, 140} = 2.40$, $P < 0.005$). These effects persisted through the 1st wk of the lizard's life (at 1 wk old, mass: $F_{15, 140} = 6.42$, $P < 0.0001$; SVL: $F_{15, 140} = 3.72$, $P < 0.0001$; tail length: $F_{15, 140} = 3.23$, $P < 0.005$). Bodily proportions also differed among lizards emerging from different nests, at hatching, and at 1 wk of age ($P < 0.05$ for all tests on residual scores for body shape). Egg survival rates also differed among nests ($\chi^2 = 59.8$, 15 df, $P < 0.0001$). However, running speeds of hatchlings were not affected (over 1 m: $F_{15, 140} = 0.72$, $P = 0.76$; over 25 cm: $F_{15, 140} = 0.97$, $P = 0.49$).

Because hatchlings from different nests were exposed to different durations of laboratory incubation, it is possible that some of these among-nest differences in hatchling phenotypes were generated during exposure to laboratory rather than field conditions. However, we found no significant correlations between the proportion of total incubation spent in the laboratory, vs. any other trait except incubation period. Hence, these analyses confirm that naturally incubated eggs from different nests produce hatchlings with significantly different phenotypes.

We now proceed to disentangle some of the factors contributing to this among-nest variation.

Are among-nest differences in hatchling phenotypes due to nest-of-origin effects, to incubation conditions, or to both?

We can answer this question by analyzing data on the translocated eggs only. We used one-factor MANOVA, with the factor being either (1) the identification number of the nest in which the eggs were originally laid; or (2) the identification number of the nest in which they incubated. If the former MANOVA provides a significant result, it means that part of the phenotypic variation among hatchling skinks is due to some factor operating prior to the time we removed the eggs from their original nests. This factor could be a ''nest-of-origin'' effect (e.g., due to genes, maternal investment patterns, or to some environmental effect that operated prior to oviposition or in the first few days after the eggs were laid). If the second MANOVA yields a significant result, it means that incubation conditions influence hatchling phenotypes.

Both tests yielded highly significant results. The first test (one-factor MANOVA on data for hatchlings from translocated eggs, with the nest of laying as the factor) showed highly significant phenotypic variation among hatchlings depending on their nest of origin (Hotelling-Lawley trace = 4.13, $F_{240, 1922} = 2.20$, $P < 0.0001$). Closer inspection of one-factor ANOVAs for each trait confirmed that these nest-of-origin effects were significant ($P < 0.05$) for most measures of hatchling size and shape, and close to significance for running speeds. However, survival rates (proportions of eggs hatching) did not depend upon their nest of origin ($\chi^2 = 23.41$, 16 df, $P = 0.10$). The analogous MANOVA for incubation effects (i.e., phenotypes of hatchlings from translocated eggs, tested against the identity of the nest in which they incubated) also showed a highly significant effect (Hotelling-Lawley trace = 4.01, $F_{240, 1922} = 2.14$, $P < 0.0001$). As for nest-of-origin effects, the variables most strongly affected by incubation environment were morphological traits rather than running speeds. Survival rates of eggs to hatching also depended on the nest to which they were translocated ($\chi^2 = 68.25$, 16 df, $P < 0.001$).

In combination, these analyses show that the among-nest differences documented from the ''natural'' situation (i.e., hatchlings emerging from the nests in which they were originally laid) are due to a combination of effects operating prior to the time the eggs were transferred in our experiment (i.e., nest-of-origin effects plus incubation conditions in the first few days of de-

velopment), and other factors that affected the eggs during incubation.

Are the thermal regimes in natural nests responsible for induction of phenotypic changes in hatchlings?

Although our analyses document an effect of incubation environment on hatchling phenotypes, we have not identified which aspects of nest environment are important in this respect. Natural nests vary in many ways, of which the two most obvious are temperature and soil moisture. However, many other variables undoubtedly differ among nests as well (e.g., trace chemicals, pesticide residues, soil characteristics such as particle size, pH) in ways that might influence hatchling phenotypes. Thus, we need to determine whether some of the incubation-induced effects are due to temperature regimes rather than to other factors.

The simplest way to see whether the thermal environment affects hatchling phenotypes is to regress phenotypic trait values (of all eggs, translocated as well as replaced) against descriptors of the thermal regime under which these eggs incubated. A significant relationship between hatchling traits and thermal variables would support the notion that nest temperatures affect hatchling phenotypes. Because laboratory experiments have shown that the phenotypes of hatchling *B. duperreyi* are influenced by thermal variability as well as by mean temperatures (Shine and Harlow 1996), we regressed hatchling traits against two descriptors of the thermal environment in each nest: the mean temperature, and an index of the variance relative to the mean (i.e., the residual score from the linear regression of the variance against the mean). This index provides a measure of the variance independent of mean nest temperature.

Our regression analyses supported the notion that incubation temperatures affect phenotypic traits of hatchlings (Fig. 1). For example, the overall mean incubation temperature significantly affected incubation period ($r = -0.74$, $n = 326$, $P < 0.0001$) and offspring size (SVL at hatching: $r = -0.11$, $n = 326$, $P = 0.05$; SVL at 1 wk: $r = -0.13$, $n = 326$, $P < 0.02$). These analyses also showed significant effects of thermal variance on hatchling traits such as incubation period ($r = -0.44$, $n = 326$, $P < 0.0001$) and hatchling SVL ($r = -0.15$, $n = 326$, $P < 0.006$; see Fig. 1). Thus, these data from hatchlings emerging from natural nests support the conclusion, from laboratory studies, that both the mean and the variance of incubation thermal regimes influence important hatchling traits such as time of emergence and size at hatching. However, our data showed no significant relationship between egg survival rates (proportion hatching) and any of our measures of the nest thermal environment (mean, variance, minimum, maximum: $P > 0.20$ for all regressions).

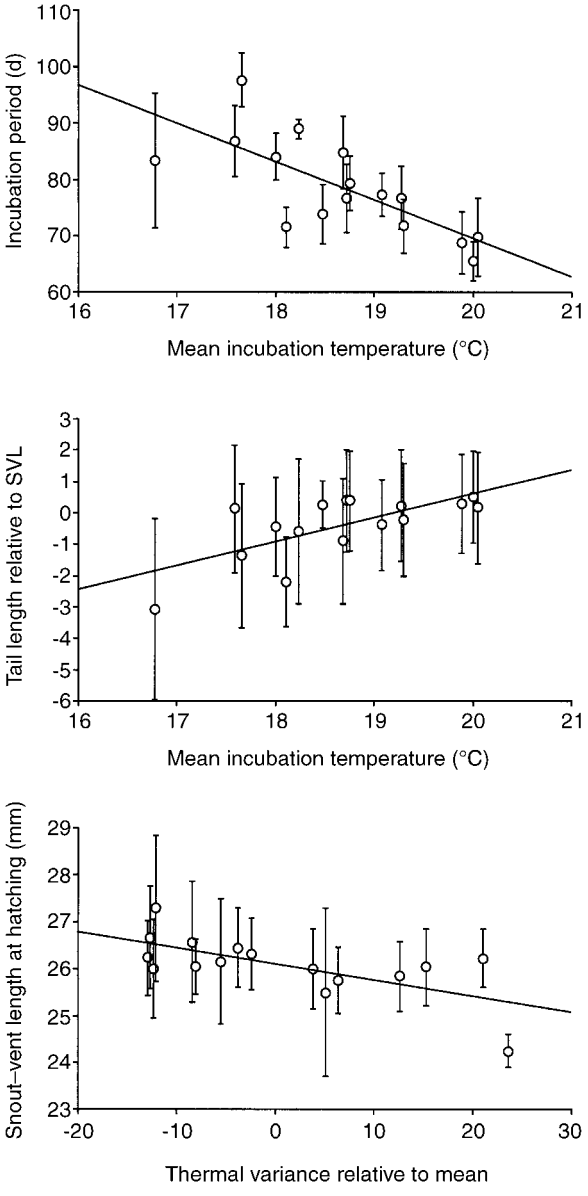

FIG. 1. The phenotypes of hatchling lizards are affected by the thermal conditions that they experience during embryogenesis. These graphs plot hatchling phenotypes (mean values ± 1 SD; one data point per nest) against thermal attributes of the nests in which they were incubated for most of development. SVL = snout–vent length; tail length relative to mean = residual score from the general linear regression of tail length against SVL; thermal variance relative to mean = residual score from the general linear regression of thermal variance against mean incubation temperature.

Do females select nest sites with thermal characteristics that optimize their own offspring's phenotypes?

Microevolutionary theory suggests that females could maximize their fitness by choosing nest sites that suit their own offspring (i.e, that take advantage of their offspring's norms of reaction: e.g., Resetarits 1996,

Roosenburg 1996). In *B. duperreyi,* for example, females with unusually slowly developing offspring might select unusually hot nests (Shine and Harlow 1996). We can test this hypothesis by examining the effect of moving eggs between nests. If mothers select nest environments relative to the norms of reaction of their offspring, then hatchlings from translocated eggs should differ consistently from their siblings that continued to develop in the original (optimal?) nest. In contrast, if hatchling phenotypes do not differ between eggs that were translocated compared to those that were replaced in the original nest, it seems unlikely that there is any such maternal matching of offspring genotypes to nest environments. Our two-factor MANOVA (factors = incubation nest number, and whether the eggs were translocated or replaced) showed that offspring phenotypes were affected by the nest in which they developed (Hotelling-Lawley trace = 3.59, $F_{210, 3782} = 4.62$, $P < 0.0001$) but not by whether or not they had been moved to that nest prior to incubation (Hotelling-Lawley trace = 0.06, $F_{14, 272} = 2.14$, $P = 0.32$). Similarly, the survival rate of eggs was not affected by whether or not they were translocated ($F_{1, 94} = 0.03$, $P = 0.86$). As well as falsifying the ''maternal-matching'' hypothesis, this result indicates that our experimental manipulation did not introduce any major artifacts; i.e., the process of translocating eggs had no discernible effect on hatchling phenotypes or the probability of successful hatching.

Another way that a female lizard could match her choice of nest site to the genetic characteristics of her offspring would be to modify her choice of nest based on the sex ratio of her offspring. This possibility arises from the fact that the phenotypes of male and female offspring respond differently to incubation temperatures (Shine et al. 1995, and see below for further evidence). However, our data show no significant correlation between the sex ratio of the eggs deposited in a nest, and the thermal characteristics of that nest (sex ratio vs. mean temperature: $r = -0.25$, $n = 16$, $P = 0.35$; vs. thermal variance: $r = -0.36$, $n = 16$, $P = 0.18$).

Do the sexes differ in their phenotypic response to incubation temperatures?

Laboratory experiments have suggested that male and female offspring may differ in their thermal optima for development, a result with implications for the evolution of temperature-dependent sex determination (Shine et al. 1995). We thus looked to see whether similar sex differences were evident under natural incubation, using two approaches.

(1) Does the effect of incubation conditions on hatchling phenotypes differ between males and females? A two-factor MANOVA with sex and incubation nest number as the factors yielded a significant interaction term (Hotelling-Lawley trace = 0.68, $F_{150, 2742} = 1.25$, $P < 0.03$), showing that the sexes responded differently to incubation regimes. This MANOVA also revealed highly significant differences in mean values for hatchling traits between the sexes (Hotelling-Lawley trace = 0.36, $F_{10, 276} = 9.92$, $P < 0.0001$) and among nests (Hotelling-Lawley trace = 0.86, $F_{150, 2742} = 1.58$, $P < 0.0001$).

(2) Does the relationship between hatchling traits and thermal regimes during incubation vary between male and female hatchlings? We used a two-factor MANCOVA, with sex as the factor and a thermal descriptor (either mean incubation temperature or variance in nest temperature) as the covariate, to investigate this possibility. Our analysis (restricted to traits correlated with incubation temperatures) showed that hatchling traits were affected by sex (against mean temperature: Hotelling-Lawley trace = 0.04, $F_{5, 311} = 2.70$, $P < 0.02$; against thermal variance: Hotelling-Lawley trace = 0.12, $F_{5, 311} = 7.63$, $P < 0.0001$) and by the thermal regime in the nest (against mean temperature: Hotelling-Lawley trace = 1.21, $F_{5, 311} = 75.16$, $P < 0.0001$; against thermal variance: Hotelling-Lawley trace = 0.26, $F_{5, 311} = 16.16$, $P < 0.0001$). More importantly, the slopes of the relationship between incubation temperature and hatchling trait value differed between the sexes (interaction term against mean nest temperature: Hotelling-Lawley trace = 0.05, $F_{5, 311} = 2.98$, $P < 0.013$; against thermal variance: Hotelling-Lawley trace = 0.04, $F_{5, 311} = 2.32$, $P < 0.05$). This result indicates that the sexes differ in their reaction norms (i.e., phenotypic responses to incubation temperatures). Hence, males and females were differentially affected by the thermal regimes they experienced during incubation. Whether or not this difference translates into the sexes having different optimum temperatures for incubation (e.g., Charnov and Bull 1977, Bull 1980, Janzen and Paukstis 1991) depends on a host of other variables, notably the way in which a particular hatchling phenotype influences lifetime reproductive success. This relationship may well differ between the sexes also (e.g., Trivers and Willard 1973, Charnov 1982, Roosenburg 1996).

What proportion of the overall phenotypic variance in hatchling reptiles within a population is generated by incubation conditions vs. nest-of-origin effects?

Several authors have speculated that a significant fraction of the total phenotypic variance among a cohort of hatchling reptiles is engendered by incubation conditions rather than genetic factors (e.g., Vleck 1988, Packard 1991, Shine 1995, Shine and Harlow 1996). Other authors have disputed this claim (see review in Packard et al. [1993]). Our experimental design allows us to quantify the relative magnitude of these two effects. In particular, the distribution of translocated eggs among different incubation environments faithfully mimicked the natural situation in this population. Thus, we can use data from the hatchlings that emerged from these translocated eggs, to quantify the relative mag-

TABLE 1. Proportion of variance in phenotypes of hatchling lizards (*Bassiana duperreyi*) due to nest-of-origin effects and incubation conditions. The proportions are derived from the relative magnitude of sums of squares in two-factor ANOVA tables, with the factors being the nest of origin, and the temperature regime under which the eggs were incubated. Only ''translocated'' eggs (i.e., those incubated in nests other than the one in which they were laid) were included in this analysis, to avoid confounding nest-of-origin and incubation-temperature effects.

Trait	Proportion of variance due to			
	Nest of origin	Incubation temperature	Interaction	Residual
Incubation period (d)	0.31	0.22	0.16	0.31
Snout-vent length (SVL; mm)				
At hatching	0.15	0.04	0.06	0.75
At 1 wk of age	0.14	0.05	0.05	0.76
Body mass (g)				
At hatching	0.34	0.03	0.07	0.56
At 1 wk of age	0.36	0.02	0.07	0.54
Tail length (mm)				
At hatching	0.26	0.10	0.07	0.57
At 1 wk of age	0.29	0.09	0.07	0.55
Body shape (mass/SVL)				
At hatching	0.36	0.02	0.06	0.57
At 1 wk of age	0.30	0.02	0.07	0.60
Relative tail length (tail/SVL)				
At hatching	0.21	0.07	0.07	0.64
At 1 wk of age	0.24	0.06	0.07	0.63
Growth rate (hatching to 1 wk)				
In SVL (mm)	0.16	0.03	0.03	0.77
In mass (g)	0.19	0.06	0.06	0.69
Running speed (m/sec)				
Over 1 m	0.16	0.02	0.10	0.73
Over 25 cm	0.13	0.01	0.09	0.77
Running speed relative to mass	0.11	0.01	0.11	0.77

nitude of their overall phenotypic variance due to (1) their nest of origin, vs. (2) the physical conditions under which they incubated.

Our approach was as follows. We carried out two-factor ANOVAs on each hatchling trait, with the factors being the nest of origin, and the mean incubation temperature (nearest integer value, used as a nominal variable). We could not use the recipient nest as the second factor, because of too many missing cells (i.e., many combinations of ''donor nest'' × ''recipient nest'' were not available in our design). Using mean nest temperature as a nominal variable (to the nearest 1°C) overcame this problem, because nest means spanned a narrow range (17.6° to 20.1°C; note that one nest averaged 16.8°C, but was omitted from our experimental treatments because of its low number of eggs) and thus, all donor nests provided eggs to recipient nests in each of these three temperature categories (18°, 19°, and 20°C). Also, this design allowed us to investigate effects of incubation temperature rather than a combination of all incubation influences. The sums of squares from the resulting ANOVA tables provide an approximation of the relative magnitude of overall phenotypic variance attributable to each of the two factors, and to the interaction between them (Snedecor and Cochran 1987).

Table 1 depicts the results of these analyses. On average, the two factors included in these ANOVAs, plus the interaction between them, explained 36% (range: 18–55%) of the variation in the phenotypic traits we measured in the young lizards. Of this ''explained'' variance, about two thirds (64%) was due to factors that operated prior to our experimental translocations (i.e., genetic constitution of the offspring, plus non-genetic nest-of-origin effects, plus effects of incubation conditions during the first few days after laying), and the other third was due to the thermal regime under which the eggs developed, and (at least as importantly) the interaction between the two factors. Although the relative importance of nest-of-origin effects, incubation-induced effects, and nest of origin × incubation environment interactions differed substantially among traits (Table 1), the clear result is that a significant proportion of the observed phenotypic variation in hatchling reptiles is present because of the diversity of thermal regimes under which the eggs incubate. According to our estimates, approximately one third of

the explained variation in hatchling phenotypes in a field population would not be expressed if all eggs were incubated under identical conditions in the laboratory.

Our analysis undoubtedly underestimates the significance of incubation environments as a contributor to variance in hatchling phenotypes, for the following reasons. (1) The variance explained by nest-of-origin effects actually includes a significant component due to thermal effects during early embryogenesis (in utero, and immediately after oviposition). Laboratory studies have shown that maternal thermoregulation prior to oviposition has a significant effect on the phenotypes of hatchling *B. duperreyi* (Shine 1995). (2) Our eggs were incubated for a considerable proportion of their development (mean = 30% of the incubation period) under identical conditions in the laboratory. (3) Our study area experienced unusually cool weather over the summer of 1995–1996, with the result that differences among nests in thermal regimes were atypically low. For example, mean nest temperatures ranged only from 17.6° to 20.1°C, whereas studies on the same population (including many of the same nest-sites) in the preceding two summers revealed mean nest temperatures of 17.3°–24.4°C (Shine and Harlow 1996). The mean daily maximum temperature of the hottest nest in 1995–1996 was only 29.7°C, vs. 38.0°C in previous years. Thus, the magnitude of incubation-induced variation in hatchling phenotypes is likely to have been much lower from the (thermally homogeneous) 1995–1996 nests than from the (thermally diverse) nests of previous years.

General Discussion

Our primary conclusion is that major results from previous laboratory incubation experiments on *Bassiana duperreyi* (Shine 1995, Shine et al. 1995, Shine and Harlow 1996) can be extrapolated to the field. More generally, our data support the proposition that incubation-induced modifications to the phenotypes of hatchling reptiles are likely to be significant in the field, as well as in the laboratory (e.g., Burger et al. 1987, Vleck 1988, Burger 1989, 1990, 1991, Packard 1991, Cagle et al. 1993, Packard et al. 1993). Our findings may be summarized as follows:

1) Hatchling phenotypes are influenced by incubation conditions as well as by nest-of-origin effects.

2) The thermal environment in natural nests (or at least, factors that are correlated with both the mean and the variance of incubation temperatures) affect hatchling phenotypes. It remains possible that these are not direct effects of the thermal environment (because many nest parameters covary, and nest temperatures are correlated with the proportional duration of incubation in the field vs. the laboratory), but the clear result is that nest characteristics related to temperature regimes influence the phenotypes of hatchling lizards.

3) The sexes respond differently to thermal conditions during incubation (i.e., they show different norms of reaction). This result is of substantial interest from the viewpoint of models for the evolution of temperature-dependent sex determination (Charnov and Bull 1977, Rhen and Lang 1995, Shine et al. 1995, Janzen 1996).

4) We found no evidence that females select nest sites with thermal characteristics that ''match'' the reaction norms displayed by their offspring. This result is not surprising, in that the matching hypothesis requires not only that a female can assess the genetic characteristics of her offspring (e.g., sex ratio, reaction norms), but also that she can predict thermal conditions in a nest over the incubation period. Given the year-to-year variation in thermal characteristics of nests, even when they are laid under the same rocks, it is difficult to understand how females could achieve this feat.

5) Thermal conditions during embryogenesis induce a significant proportion of the overall phenotypic variance in hatchling lizards. Quantitative generalizations in respect to this issue are likely to be difficult to make, because the exact magnitude of incubation-induced variance will depend upon species-specific organismal traits (e.g., the degree of sensitivity of embryogenesis to incubation conditions) as well as site-specific nest characteristics (e.g., means and variances of nest temperatures and water potentials) that may well vary appreciably over short distances and brief spans of time.

Because of these sources of variation, we will need studies on many other systems before we can assess the generality of our results from *B. duperreyi*. We have the data, however, to calculate proportional variances due to incubation effects in one other reptile species. A recent study on water pythons (*Liasis fuscus*) by Shine et al. (1997) provides information on clutches incubated under the thermal regimes characteristic of natural nests (as simulated in the laboratory). In a field population in tropical Australia, only two types of nest sites are readily available to these snakes: soil burrows with high constant temperatures, or tree-root-bole nests that are cooler, and show greater thermal fluctuation (Shine et al. 1997). Approximately half of the female pythons lay their eggs in nests of each type, and Shine et al. (1996) split captive-laid clutches and incubated half of each clutch at each thermal regime. From two-factor ANOVAs, with clutch number and incubation treatment as the factors, we have calculated the proportional variance in hatchling phenotypes (size, shape, locomotor speed) due to clutch effects, incubation treatments, and the interaction between these two factors. The result is broadly similar to that which we have obtained for *Bassiana duperreyi*, despite the enormous difference in the study species (adult body masses <7 g vs. >1 kg), their habitats (alpine meadows vs. tropical floodplain), and the thermal regimes in natural nests in the two areas (mean incubation temperatures of $\sim$20°C vs. 32°C). In the pythons, $\sim$60% of the total

variance was explicable by the factors in the ANOVAs. Of this ''explained'' variation, about a third was due to a combination of incubation effects (15%) and the interaction between clutch effect and incubation treatment (24%). Hence, in both systems, ''maternal'' effects (including effects of incubation conditions when the eggs were in utero, prior to oviposition) accounted for about twice as much variance as the direct influences of the thermal environment on the embryo after oviposition.

This topic has interesting biological implications, and offers great potential for additional study. For example, suitable nest sites may be limited in many cold environments, resulting in a high frequency of communal oviposition in the few available sites. If within-nest thermal variance is low in such sites, incubation-induced variance in hatchling phenotypes may be minimal, thereby reducing the overall phenotypic variance among offspring, and increasing the relative magnitude of genetic contributions to that variance. With a lower phenotypic variance and a stronger genetic underpinning to that variance, such a system may respond quite differently to natural selection on offspring phenotypes than would a nearby population faced with a greater range of potential nest sites.

Many questions thus remain about the role of incubation-induced modifications to phenotypes of hatchling reptiles. We still know very little about the relative magnitude of these effects in natural populations, the degree of persistence of these effects through an organism's ontogeny, or the ways in which such modifications may influence lifetime reproductive success. These questions remain as major challenges for future work.

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