Biological Journal of the Linnean Society (2001), *74:* 339–350. With 1 figure
doi: 10.1006/bijl.2001.0581, available online at http://www.idealibrary.com on IDEAL®

Phenotypic variation in an oviparous montane lizard (*Bassiana duperreyi*): the effects of thermal and hydric incubation environments

THOMAS FLATT[1]*, RICHARD SHINE[2], PEDRO A. BORGES-LANDAEZ[2] and SHARON J. DOWNES[2]†

[1] *Zoology Institute, University of Basel, Rheinsprung 9, 4051 Basel, Switzerland*
[2] *School of Biological Sciences, University of Sydney, NSW 2006, Australia*

Received 16 March 2001; accepted for publication 25 June 2001

Recent studies have shown that incubation temperatures can profoundly affect the phenotypes of hatchling lizards, but the effects of hydric incubation environments remain controversial. We examined incubation-induced phenotypic variation in *Bassiana duperreyi* (Gray, 1938; Sauria: Scincidae), an oviparous montane lizard from south-eastern Australia. We incubated eggs from this species in four laboratory treatments, mimicking cool and moist, cool and dry, warm and moist, and warm and dry natural nest-sites, and assessed several morphological and behavioural traits of lizards after hatching. Incubation temperature influenced a lizard's hatching success, incubation period, tail length and antipredator behaviour, whereas variation in hydric conditions did not engender significant phenotypic variation for most traits. However, moisture affected incubation period slightly differently in males and females, and for a given snout–vent length moisture interacted weakly with temperature to affect lizard body mass. Although incubation conditions can substantially affect phenotypic variation among hatchling lizards, the absence of strong hydric effects suggests that hatchling lizards react less plastically to variation in moisture levels than they do to thermal conditions. Thus, our data do not support the generalization that water availability during embryogenesis is more important than temperature in determining the phenotypes of hatchling reptiles.
© 2001 The Linnean Society of London

ADDITIONAL KEY WORDS: reptiles – hatchling – phenotypic plasticity – reaction norm – temperature – water potential – sexual dimorphism – life history – phenotype.

INTRODUCTION

Environmentally induced phenotypic variation (phenotypic plasticity) has been well documented in a variety of organisms (Sultan, 1987; Travis, 1994; Gotthard & Nylin, 1995), but its ecological and evolutionary significance remains obscure (Via *et al.*, 1995). Although plastic responses to environmental conditions may occur at any stage of an organism's life-cycle, phenotypic plasticity during early phases of ontogeny may be of particular importance because embryogenesis and birth (or hatching) are likely to be under strong selection (Lindström, 1999). For instance, environmental conditions experienced during early ontogeny can significantly affect developmental trajectories and embryonic growth rates (Arnqvist & Johansson, 1998). Particularly in oviparous organisms, where a large proportion of development occurs outside the mother's body, the incubation environments experienced by eggs can profoundly affect offspring phenotypes.

In oviparous reptiles, eggs develop in nests that can vary for thermal, hydric, edaphic and biotic factors, creating distinct incubation environments (Packard & Packard, 1988). Recent studies have focused on two such features of reptilian incubation environments: moisture and temperature. However, most work on the phenotypic effects of moisture has been conducted on turtles (Packard, 1991), whereas studies concerning temperature usually focussed on snakes and lizards

* Corresponding author. Present address: Department of Biology, Unit of Ecology and Evolution, University of Fribourg, Chemin du Musée 10, 1700 Fribourg, Switzerland.
† Present Address: School of Botany and Zoology, Australian National University, Canberra ACT 0200, Australia.
E-mail: thomas.flatt@unibas.ch

0024–4066/01/110339+12 $35.00/0
© 2001 The Linnean Society of London

(Deeming & Ferguson, 1991). For instance, variation in moisture profoundly affects egg survivorship, incubation period, hatchling size and locomotor performance in the common snapping turtle (*Chelydra serpentina*) (Packard, 1999). Similarly, variation in temperature can affect egg survivorship, incubation period and hatchling running speed in the common wall lizard, *Podarcis muralis* (Van Damme *et al.*, 1992). Recent studies on hatchling reptiles from natural nests confirmed the ecological relevance of previous laboratory findings (Cagle *et al.*, 1993; Weisrock & Janzen, 1999).

Few studies have investigated the combined effects of incubation temperature and moisture (e.g. Muth, 1980; Packard *et al.*, 1987; Phillips & Packard, 1994). In natural nests, however, these physical factors may be correlated and exert combined effects on an organism's phenotype (Packard & Packard, 1988). Thus, temperature and moisture must be considered simultaneously to understand their relative importance for determining the phenotypes of hatchling reptiles. Laboratory experiments conducted predominantly on turtles suggest that eggs incubated in cool, moist conditions have a greater survivorship and incubation period, and produce larger hatchlings than do eggs incubated in warm, dry conditions (Packard, 1999). In contrast, the few studies conducted on lizards usually failed to find any significant effects of moisture or interaction between temperature and moisture on hatchling phenotypes (e.g. Ji & Braña, 1999). This failure has been attributed by some workers to weaknesses in experimental design, e.g. unrealistic moisture conditions or unassessed sources of phenotypic variation (e.g. among clutches, nests of origin), leading to 'experimental' noise and thereby preventing the detection of moisture effects (e.g. Packard, 1991). Thus, powerful factorial experiments using realistic (both thermal and hydric) incubation conditions and accounting for all major sources of phenotypic variation are needed. Furthermore, most studies have been restricted to phrynosomatid and iguanid lizards. To obtain a general perspective on this phenomenon, studies must be conducted on other lizard groups.

Here we examine the effects of thermal and hydric incubation environments on phenotypic variation among hatchlings of the montane scincid lizard *Bassiana duperreyi*. In contrast to previous laboratory work (reviewed in Deeming & Ferguson, 1991; Packard, 1991), temperature and moisture regimes were applied that mimicked those found in natural nests. Our factorial experimental design (simulating cool–moist, cool–dry, warm–moist and warm–dry nests) allowed us to disentangle the relative importance of temperature and moisture and their interaction in affecting variation in offspring traits. We examined incubation-induced effects on traits that are likely to be important components of fitness: (1) incubation period, (2) hatching success; (3) hatchling size and (4) hatchling performance, i.e. running speed and antipredator behaviour. Because of the scarcity of data on gender × incubation effects on hatchling phenotypes (Elphick & Shine, 1999), we also investigated whether or not females and males respond differently to thermal and hydric conditions experienced during incubation.

MATERIAL AND METHODS

STUDY SPECIES

The montane three-lined skink *Bassiana duperreyi* is a medium-sized (to 80 mm snout–vent length) oviparous diurnal lizard widely distributed in the montane grasslands of sub-alpine south-eastern Australia (Cogger, 1992). Adult females deposit a single clutch (range = 3–7 eggs; Greer, 1982) annually under rocks or logs and often use communal nest sites. In the large majority of nests, eggs are buried into the soil at a depth of 2.7 to 3.7 cm under rocks and logs; however, eggs are sometimes laid directly on the soil surface under logs (e.g. Shine, 1999). We chose *B. duperreyi* as a study system for three reasons. First, long-term monitoring has documented significant spatio–temporal thermal variation among nests, so that biologically realistic warm and cool incubation regimes can be simulated in the laboratory (Shine & Harlow, 1996). Second, laboratory and fieldwork on this species has demonstrated that thermal variation within the natural range of temperatures can substantially modify hatchling phenotypes (e.g. Shine & Harlow, 1996; Shine, Elphick & Harlow, 1997; Elphick & Shine, 1998). However, the phenotypic effects of moisture or of interactions between moisture and temperature have not been investigated so far. Third, *B. duperreyi* has genotypic sex determination (GSD, Donnellan, 1985), and incubation temperature does not affect sex ratios over the range of temperatures used in our previous studies (Shine, Elphick & Harlow, 1995; Elphick & Shine, 1999). However, incubation temperature has been reported to interact with offspring gender in determining some phenotypic traits among hatchlings (Shine *et al.*, 1995; Elphick & Shine, 1999). Our experiment allowed us to examine such gender × incubation environment interactions.

EGG COLLECTION AND INCUBATION

Egg laying is synchronous among female *B. duperreyi* from our study population in the Brindabella Ranges, 40 km west of Canberra in the Australian Capital Territory (Pengilley, 1972). Fieldwork was timed to coincide with the beginning of egg laying. In late December 1998, we collected 210 recently deposited eggs from 18 natural communal nests. It was usually

not possible to recognize individual clutches but we estimate 44 clutches based on a mean clutch size of 4.8 eggs (Greer, 1982). Eggs were probably laid up to 1 week before collection (based on their incubation periods and our regular inspection of nests).

Eggs were transported to our laboratory at the University of Sydney in moist vermiculite (−200 kPa duelit grade 2 vermiculite, L. and A. Fazzini, NSW, Australia). Upon arrival, they were weighed to the nearest 0.001 g on a top-loading balance and transferred individually to 64-ml glass jars filled with vermiculite. By distributing eggs evenly among our four incubation treatments (cool–dry, cool–moist, warm–dry, warm–moist), any consistent phenotypic differences among treatments should reflect incubation conditions rather than (genetic, maternal or environmental) nest of origin effects. The mass of eggs allocated to different treatments was not significantly different (two-way ANOVA, $df = 1, 200$, in all cases $P > 0.24$).

Eggs were kept half-buried in dry or moist vermiculite, either in warm or cool conditions, throughout the incubation period. Dry vermiculite was set at a water potential of −750 kPa, whereas moist vermiculite was at −200 kPa (84.6% and 120% water by dry mass vermiculite, respectively). The amount of water needed to achieve dry and moist conditions was determined from a previously established standard curve of water content versus water potential. To quantify whether experimental moisture levels approximated those in qualitatively moist and dry natural nests, 32 soil samples from 16 nests containing developing eggs were obtained in February 1998. Soil water potential was then determined by using a filter-paper method (Hamblin, 1981). Water levels in natural nests ranged from moist (−170 kPa) to dry (−1200 kPa) conditions (mean = −662.5 kPa, SE = 69.6, $N = 16$). Thus, the moisture levels applied to eggs in our experiment were well within the range experienced by eggs in nature. Moisture loss throughout incubation was minimized by sealing jars with plastic wrap. We did not attempt to regularly replace water lost during incubation because a constant water potential during incubation is unlikely to reflect the situation in natural nests where water potentials are expected to change over time. To examine temporal variation in moisture levels, water potentials of vermiculite from each incubation treatment ($N = 24$ and $N = 26$ jars for dry and moist treatments, respectively) were remeasured at the end of the experiment. Each sample was weighed, oven-dried for 72 h at 60°C, reweighed to determine its gravimetric water content, and its water potential calculated from the standard curve. The moisture of vermiculite declined in both treatment groups during the experiment. However, at the end of the study the vermiculite was still considerably drier in the dry treatments (mean ± SE [kPa] = −1467 ± 58.9, $N = 24$) than in the moist treatments (−314.4 ± 11.0, $N = 26$; two-way ANOVA, ln (data): $F_{1,46} = 689$, $P < 0.01$). The same analysis showed no significant difference in the average moisture of vermiculite between the cool and warm treatments ($F_{1,46} = 0.44$, $P = 0.5$) and no significant interaction between incubation temperature and moisture ($F_{1,46} = 0.55$, $P = 0.46$). Thus, although moisture levels changed during the experiment, dry conditions remained qualitatively dry relative to moist conditions at both incubation temperatures. Warm versus cool nest conditions were simulated using two Clayson 10-step programmable incubators. One incubator mimicked warm nests by undergoing 24-h sinusoidal fluctuations around a mean temperature of 22°C (amplitude ±5°C), whereas the other incubator was set to simulate cool natural nests, fluctuating around 18°C (±5°C). Mean temperatures experienced by eggs in natural nests span a range between 17.3°C and 24.4°C (Shine & Harlow, 1996). Therefore our experimental thermal regimes closely approximate natural conditions. However, for logistic reasons, we were unable to replicate incubators for each temperature. Thus, temperature effects are potentially confounded by other putative incubator effects (see Hurlbert, 1984). Although we cannot rule out this possibility, we think that this is extremely unlikely for two reasons. First, all previous studies on *B. duperreyi* conducted in our laboratory clearly show that thermal effects on phenotypic traits are qualitatively reproducible among experiments. Phenotypic effects of temperature are consistent if different incubators of the same trademark are used to mimic qualitatively warm or cool nests (e.g. Shine, 1995; Shine & Harlow, 1996; Elphick & Shine, 1998). Second, the direction and magnitude of most temperature effects reported here is consistent with those in our previous studies. We therefore interpret differences among hatchlings from different incubators as temperature effects. The position of the jars was changed weekly to homogenize the effects of any temperature gradients within the incubators. Incubators were checked daily for hatchlings.

MAINTENANCE OF LIZARDS

Hatchling lizards were kept, in groups of four or five individuals, in plastic boxes ($22 \times 13 \times 7$ cm^3) containing a wood shelter and filled to 1 cm with soil. Boxes were housed at 18 ± 1°C, and heating strips (attaining 34°C) underneath one end of the container allowed hatchlings to thermoregulate for 10 h a day (light [warm]:dark [cool] cycle = 10:14 h). During our studies on *B. duperreyi* we have never observed behavioural interference amongst hatchling lizards for a heat source. All lizards housed in the same box therefore were highly likely to have simultaneous and unrestricted access to all regions along the thermal

gradient. However, for logistic reasons we were unable to record individual thermal preferences. Thus, we do not know whether hatchlings from different treatments had consistently different thermal preferences and whether the temperature experienced by an individual influenced its performance in the running trials. Lizards were provided with ad libitum water and fed a standardized amount of crickets (*Acheta domesticus*) or mealworms (larvae of *Tenebrio molitor*) four days prior to their running trial and twice a week thereafter. After completing the experiment, all lizards were released in the study area, close to their nest of origin.

HATCHLING TRAITS

On the birth day of each hatchling, the presumed incubation period (=date of hatching − date of start of incubation experiment, days) was calculated, sex was determined by manual eversion of hemipenes, and hatchling body mass (±0.01 g), snout–vent length (SVL, ±0.05 mm) and tail length (±0.05 mm) were measured. Unfortunately, we were unable to measure growth rates because of logistic restrictions (see Elphick & Shine, 1999 for incubation-induced effects on growth rates in *B. duperreyi*). To assess hatching success, the experiment continued until all eggs hatched or were found to be dead (as indicated by fungal growth on the outside of the egg and confirmed by dissection).

At 7 days of age, a lizard's running speed was measured along a 1 m long and 4 cm wide electronic racetrack. Lizards were acclimated to a room temperature of 25°C (the normal activity temperature for *B. duperreyi*, Shine, 1983) for at least 30 min prior to a trial. Individuals were then placed in the holding area of the raceway before being released and allowed to run the 1-m distance. In all cases lizards were chased with an artist's paintbrush. Each individual was run three times, with 10 min of rest between successive trials. Running speeds (m/s) were determined with an infrared timing device connected to the track, using photocells at 25-cm intervals along the runway. From these data, mean burst speed (fastest speed over any 25-cm segment, m/s) and mean sprint speed (mean running speed over 1 m, m/s) were calculated for each individual.

In the running trials we also recorded whether a hatchling stopped during a trial, reversed direction, and then ran back past the paintbrush; some individuals also vertically raised and wriggled their tails. This behaviour was previously reported in hatchling *B. duperreyi* and other lizard species (Elphick & Shine, 1998), and is postulated to function as an antipredator tactic (Shine, 1995). Because our main focus was on measuring running speeds rather than on antipredator behaviour, we did not measure parameters such as the latency and duration of the behaviour. We analysed the frequency of the antipredator behaviour in a given treatment by treating the behaviour as a dichotomous variable (e.g. present versus absent in a set of three trials per lizard). However, we did not analyse the number of trials in which a lizard performed the behaviour or the number of times the behaviour was performed in a given trial because (i) the behaviour is usually highly repeatable among trials and (ii) during a given trial a lizard has usually only once the opportunity to perform the behavioural display.

STATISTICAL ANALYSIS

Statistical analyses were performed by following procedures described in Sokal & Rohlf (1995) and using the programs SAS v.6.12 (SAS Institute, 1989) and SYSTAT 5.1 (SYSTAT, Inc., Evanston, IL, USA; Wilkinson, 1989).

Because many of the hatchling traits measured in this study were likely to be correlated, we analysed the dependent variables using MANOVA before proceeding to ANOVAs for the individual traits. To test for an overall effect of incubation conditions, the dependent variables (incubation period, hatchling mass, SVL, tail length, burst speed, sprint speed) were analysed using a four-way MANOVA with temperature (i.e. incubator), moisture, sex and nest of origin as factors, considering main effects only.

For each of the dependent variables (see above), we then performed a four-way ANOVA using procedure GLM (SAS Institute, 1989) with temperature (i.e. incubator), moisture, sex and nest of origin as factors. Assumptions of ANOVA were tested using the SAS program macro HOMOVAR and procedure UNIVARIATE (SAS Institute, 1989). If assumptions were violated, we transformed data as necessary. Because we could not disentangle with our design whether and to what extent nest of origin effects consisted of genetic effects, maternal effects and environmental effects experienced by eggs prior to transfer to the laboratory, we decided not to make inferences about this composite source of variation. Nest of origin was included only to correct for variation among nests and was therefore taken as a fixed factor. Thus, we only report results for the main effects and interactions of temperature, moisture and sex (see Results), although variation among nests of origin was accounted for in all ANOVA models presented (and was significant in all cases except for burst speed; results not shown). All interaction terms with $P>0.2$ were eliminated from the full ANOVA model to increase the power to detect interactions of lower order or main effects. The unequal distribution of eggs among treatments with respect to nest of origin resulted in empty cells. We therefore used type IV

sums of squares (SSQ). As pointed out by Shaw & Mitchell-Olds (1993), an ANOVA using type IV SSQ cannot be considered a complete analysis because alternative parametric hypotheses may exist. However, we note that performing an orthogonal fixed-factorial three-way ANOVA (using type III SSQ) without correcting for variation among nests of origin yielded qualitatively very similar results with respect to effects due to temperature, moisture and sex as did the four-way ANOVA (using type IV SSQ; results not shown). Because we performed six ANOVAs (one for each trait), we corrected the P values for multiple comparisons using Bonferroni adjustment.

Although behavioural interference amongst lizards housed together in a single box before the running trials was unlikely, it was possible that box effects would confound any incubation effect on running speeds. Including box as an additional independent variable in our four-way ANOVA models would, however, absorb a substantial number of degrees of freedom and reduce the power of the statistical models to an undesirable level. We therefore computed residuals from our four-way ANOVAs on running speeds and analysed these residuals in a one-way ANOVA using box as a factor. For both burst and sprint speed, box effects were not significant ($F_{1,140}=0.9$, $P=0.66$; $F_{1,140}=1.15$, $P=0.28$), suggesting that box effects are unlikely to be confounding.

Because body mass, tail length and running speed correlated significantly with SVL, we performed ANCOVAs to test whether the effects observed in ANOVAs were still significant after accounting for SVL. The assumption of homogeneity of slopes was tested as an interaction between the main factors (or interactions) and the covariate in the linear model. All interaction terms with $P>0.2$ were eliminated from the full ANCOVA model.

Log-linear models (LLM, multidimensional contingency table analysis) were used to test for significant interactions between the two independent categorical variables temperature (warm, cool) and moisture (moist, dry) and a third dependent variable, hatching success (category hatched: yes, no) or sex (category sex: male, female). Log-linear modelling is a statistical method for analysing categorical data (Sokal & Rohlf, 1995). The aim is to find the simplest model in a hierarchy of models, i.e. the model containing the fewest number of parameters that does not deviate significantly from a perfect fit (as measured by a G test). To find the simplest model in the hierarchy, we followed Goodman's (1971) stepwise procedure (Lee, 1978).

Standard deviations for proportions (hatching success, antipredator behaviour) were calculated as binomial standard errors.

RESULTS

OVERALL PHENOTYPIC EFFECTS OF INCUBATION CONDITIONS

Because hatchling traits were correlated (Table 1), we used MANOVA to analyse the overall phenotypic pattern of incubation effects. Incubation temperature, sex, and nest-of-origin affected the overall hatchling phenotype significantly (MANOVA, $F_{6,115}=1344.1$, 9.4, and 2.5, respectively; Wilks' $\lambda<0.001$ in all cases). In contrast, moisture did not affect a hatchling lizard's overall phenotype (MANOVA, $F_{6,115}=1.05$, Wilks' $\lambda=0.4$).

INCUBATION PERIOD

The mean incubation period of eggs was significantly different among treatments: cool-incubated hatchlings had a 21% longer incubation period than did warm-incubated hatchlings (Table 2, Fig. 1A). Moisture affected incubation period differently in hatchling male and female lizards (Table 2). However, the effects were only minor: dry-incubated males had a 0.9% longer incubation period than dry-incubated females whereas moist-incubated females had a 0.9 % longer incubation period than dry-incubated females ($t=2.8$, $df=70$, $P<0.05$, and $t=2.9$, $df=68$, $P<0.05$, respectively). These contrasts remained significant after Bonferroni adjustment.

HATCHING SUCCESS

Eggs incubated in warm conditions were 1.3 times more likely to hatch than were those incubated in cool conditions (Fig. 1B), and incubation treatments did not affect the sex ratio of hatchling lizards at birth (LLM, simplest model: $G^2=0.19$, $df=3$, $P=0.98$, and $G^2=0.25$, $df=4$, $P=0.99$; in both cases no significant deviation from perfect fit). The sex ratio at birth, summed over all treatments, was not significantly different from 1:1 (females:males = 84:79; binomial test, $P=0.69$).

HATCHLING MORPHOLOGY

Lizards hatched from eggs incubated under different thermal and hydric conditions did not differ significantly in mass, and body mass did not differ between the sexes (Table 2, Table 3). Similarly, a hatchling lizard's SVL was not significantly affected by incubation temperature or moisture. However, the sex of a newborn lizard significantly affected its SVL: females were 1.7% larger than males (Table 2, Table 3). Because a hatchling's body mass was significantly correlated with its SVL, we performed an ANCOVA on body mass, using SVL as the covariate. For a given SVL, incubation temperature and moisture interacted

Table 1. Correlation matrix for the hatchling traits incubation period, body mass, SVL, tail length, burst and sprint speed. Upper values refer to Pearson's correlation coefficients, and lower values refer to *P* values. Significant *P* values (<0.05) are presented in boldface

	Incubation period (d)	Hatchling mass (g)	SVL (mm)	Tail length (mm)	Burst speed (m/s)	Sprint speed (m/s)
Incubation period (d)	1.00	—	—	—	—	—
	0.00	—	—	—	—	—
Hatchling mass (g)	0.10	1.00	—	—	—	—
	0.24	0.00	—	—	—	—
SVL (mm)	0.05	0.61	1.00	—	—	—
	0.60	**<0.01**	0.00	—	—	—
Tail length (mm)	−0.50	0.43	0.36	1.00	—	—
	<0.01	**<0.01**	**<0.01**	0.00	—	—
Burst speed (m/s)	−0.05	0.18	−0.08	0.12	1.00	—
	0.58	**<0.05**	0.35	0.15	0.00	—
Sprint speed (m/s)	−0.19	0.09	−0.12	0.15	0.84	1.00
	0.03	0.31	0.17	0.08	**<0.01**	0.00

Table 2. Summary statistics for four-way ANOVAs examining the effects of incubation temperature, moisture, hatchling sex and next of origin on incubation period, morphology and locomotor performance of *B. duperreyi*. Results for effects due to nest of origin are not given here (see Methods). Upper values refer to *F* values, and lower values refer to *P* values. Significant *P* values (<0.05) are presented in boldface. Interaction terms with *P*>0.2 were eliminated from the model. If corrected for multiple comparisons using Bonferroni adjustment, the sex difference in tail length is not significant. Abbreviations: T = temperature, M = moisture, S = sex, ln = natural logarithm, sqrt = square root, SVL = snout–vent length

	Main effects			Interactions			
Dependent variable (*df*)	T	M	S	TxM	TxS	MxS	TxMxS
ln (incubation period) (days)	6771.11	1.23	1.19	—	—	8.61	—
(1, 102)	*P*<**0.001**	*P*=0.27	*P*=0.28	*P*>0.2	*P*>0.2	*P*<**0.005**	*P*>0.2
Hatchling mass (g)	0.43	1.25	2.41	—	—	—	—
(1, 120)	*P*=0.51	*P*=0.27	*P*=0.12	*P*>0.2	*P*>0.2	*P*>0.2	*P*>0.2
sqrt (svl) (mm)	0.01	0.47	32.85	—	—	—	—
(1, 120)	*P*=0.90	*P*=0.49	*P*<**0.001**	*P*>0.2	*P*>0.2	*P*>0.2	*P*>0.2
Tail length (mm)	49.45	1.32	4.24	—	—	—	—
(1, 81)	*P*<**0.001**	*P*=0.25	*P*<**0.05**	*P*>0.2	*P*>0.2	*P*>0.2	*P*>0.2
ln (burst speed) (m/s)	3.12	0.25	1.56	—	—	—	—
(1, 120)	*P*=0.08	*P*=0.62	*P*=0.21	*P*>0.2	*P*>0.2	*P*>0.2	*P*>0.2
Sprint speed (m/s)	0.12	0.00	0.00	—	—	—	—
(1, 107)	*P*=0.72	*P*=0.96	*P*=0.99	*P*>0.2	*P*>0.2	*P*>0.2	*P*>0.2

to affect body mass (intercepts test, $F_{1,100}=4.61$, $P<0.05$). Cool–dry incubated lizards were 3.2% heavier than cool–wet incubated lizards and 2.7% heavier than warm–dry incubated lizards ($t=2.4$, $df=51$, $P<0.05$, and $t=2.2$, $df=70$, $P<0.05$, respectively). Although the interaction was significant, these contrasts were not significant after Bonferroni adjustment. The relationship between a hatchling's body mass and its SVL differed between the sexes (homogeneity of slopes, $F_{1,100}=5.5$, $P<0.05$). Hatchlings incubated under warm conditions had 9.5% longer tails than lizards that developed under cool conditions (Table 2, Fig. 1C). Males had 2.1% longer tails than females (Table 2: $P=0.04$, marginally significant; Fig. 1C), but this effect was not significant when Bonferroni adjustment was applied. However, both effects were significant in an ANCOVA on tail length, using SVL as the covariate. For a given SVL, both temperature and sex affected tail length (intercepts test, $F_{1,102}=108.2$, $P<0.001$ and $F_{1,102}=17.9$, $P<0.001$, respectively).

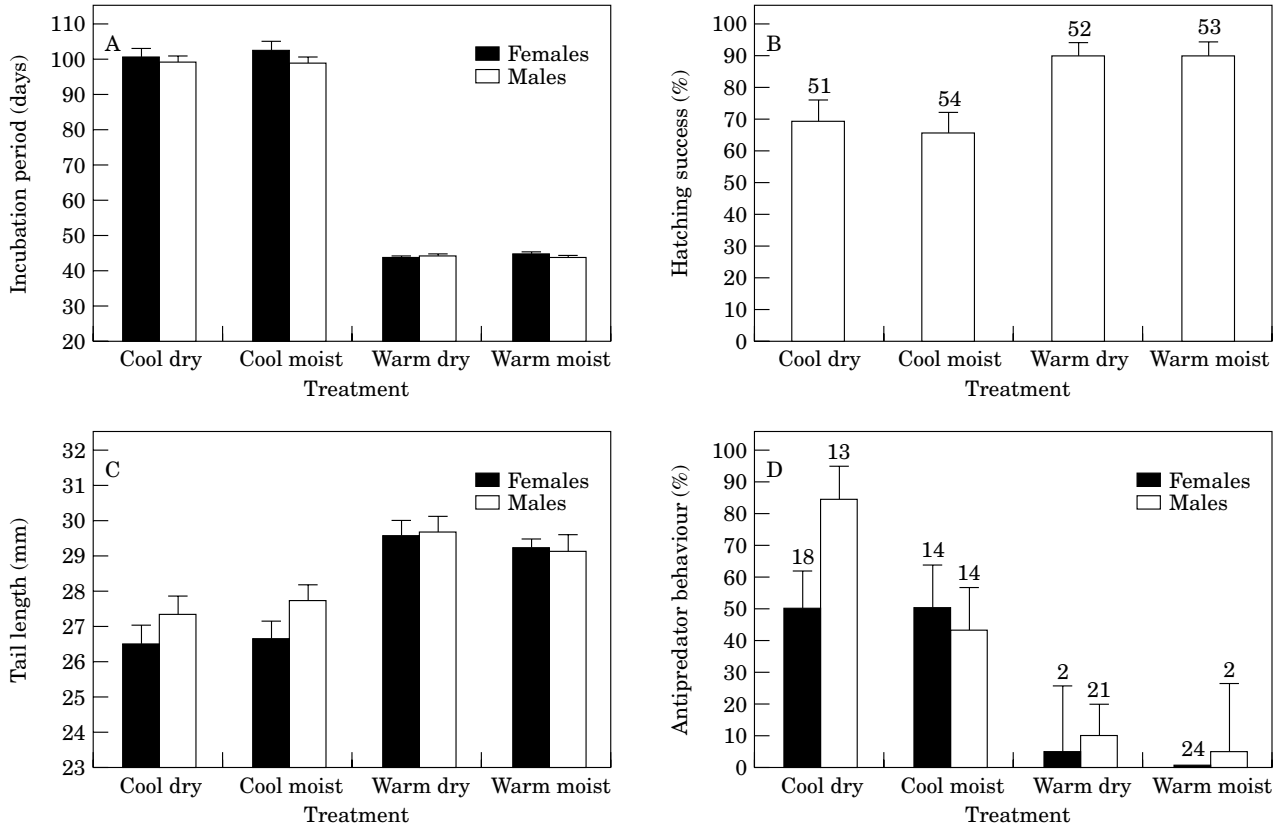

Figure 1. Effects of hatchling sex and incubation treatment (cool–dry, cool–moist, warm–dry, warm–moist, see Methods for further details) on incubation period, hatching success, tail length and antipredator behaviour of hatchling *B. duperreyi*. (A) Means and standard errors (error bars) for incubation period (days). See Table 2 for sample sizes. (B) Percentage hatching success. Note that we do not distinguish between the sexes because unhatched lizards were not sexed. The total number of observations in each group is given on top of each bar. Error bars indicate binomial standard errors. (C) Means and standard errors (error bars) for tail length (mm). See Table 2 for sample sizes. (D) Percentage antipredator behaviour ('raised tail wag' and 'turn around') displayed during running trials. The total number of observations (i.e. running trials) in each group is given on top of each bar. Error bars indicate binomial standard errors. Note that among warm–moist females there was no case of antipredator display.

HATCHLING PERFORMANCE

A lizard's running speed (over 0.25 m and 1 m) was not significantly modified by incubation temperature, moisture or sex (Table 2, Table 3). However, the relationship between a hatchling's sprint speed and SVL differed between the sexes (ANCOVA, homogeneity of slopes, $F_{1,87}=11.5$, $P<0.01$). Cool-incubated lizards displayed the antipredator behaviour during running trials 12 times more often than did warm-incubated hatchlings (Fisher's exact $P<0.001$; Fig. 1D).

DISCUSSION

PHENOTYPIC EFFECTS OF MOISTURE

To test for phenotypic effects of moisture we assessed several hatchling lizard traits under two hydric conditions that were well within the range of moisture levels observed in natural nests. The main result of our study is that we were unable to detect any main effects of moisture on phenotypic traits of hatchlings. However, moisture interacted with sex in affecting incubation period, and for a given SVL body mass was affected by an interaction between moisture and temperature. Although both interactions were significant, their effects were only minor (see discussion below). Although some studies have shown that moisture can affect a hatchling lizard's phenotype (Packard, Packard & Boardman, 1980; Overall, 1994; Phillips & Packard, 1994), others have failed to find phenotypic variation among hatchling lizards incubated under different moisture levels (Tracy, 1980, water potentials ranging from −200 to −590 kPa; Alberts *et al.*, 1997: −150 to −1100 kPa; Ji & Braña, 1999: 0 to −220 kPa). The differences between these studies are unlikely to be solely due to differences in experimental design,

Table 3. Effects of hatchling sex and incubation treatment (cool–dry, cool–moist, warm–dry, warm–moist; see Methods for further details) on mass, snout–vent length, burst speed and sprint speed of hatchling *B. duperreyi*. The table shows mean ± SE of untransformed data. Abbreviations for treatment combinations: C = cool, W = warm, D = dry, M = moist, f = female, m = male. The effects of incubation treatment and sex on other traits (incubation period, hatching success, tail length, antipredator behaviour) are displayed in Figure 1

	Treatment combination							
	C and D		C and M		W and D		W and M	
Variable	f	m	f	m	f	m	f	m
Sample size	15	13	11	14	22	22	22	22
TRAITS								
Hatchling mass (g)	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.005	0.27 ± 0.004	0.26 ± 0.005
Snout–vent length (mm)	25.5 ± 0.17	24.7 ± 0.16	25.8 ± 0.14	25.1 ± 0.22	25.6 ± 0.22	24.8 ± 0.22	25.7 ± 0.11	24.6 ± 0.20
Burst speed (m/s)	0.28 ± 0.02	0.32 ± 0.02	0.30 ± 0.02	0.29 ± 0.02	0.30 ± 0.01	0.35 ± 0.02	0.31 ± 0.02	0.33 ± 0.01
Sprint speed (m/s)	0.23 ± 0.02	0.26 ± 0.01	0.25 ± 0.02	0.24 ± 0.02	0.23 ± 0.01	0.26 ± 0.01	0.24 ± 0.01	0.26 ± 0.01

e.g. in the moisture regimes used (e.g. Packard *et al.*, 1980: water potentials ranging from −100 to −450 kPa; Phillips & Packard, 1994: −150 to −1100 kPa; this study: −200 and −750 kPa).

In contrast to lizards, a consistent trend is that turtles incubated in moist environments mobilize nutrients from the yolk quicker, grow faster, hatch later, are larger at birth and run faster than siblings incubated under drier conditions (Cagle *et al.*, 1993; Packard, 1999). In combination, these studies suggest that chelonians may be more sensitive to variation in moisture conditions than lizards and that hatchling responses to moisture might be more variable in lizards than in turtles. This discrepancy is difficult to explain. The contrasting results might reflect differences in nesting behaviour (as well as differences in eggshell morphology; Packard & Packard, 1988) between these two groups of reptiles as well as among different lizard species. Most turtles deposit eggs in a nest cavity in a humid zone of the soil, whereas oviposition behaviour is extremely variable among different lizard species (Packard & Packard, 1988). For example, small scincid lizards often deposit eggs beneath surface objects, but many iguanid lizards dig chambers as nests for eggs. Eggs of *B. duperreyi* are buried close to the soil surface at a depth of 2.7–3.7 cm, under small rocks and logs (Shine, 1999). Eggs located close to the surface are likely to be exposed to extremely dry conditions when the uppermost layer of soil dries after a period of rainfall. In contrast, eggs buried in deeper layers or deposited in a nest chamber are likely to experience more favourable hydric conditions (Ackerman, 1991; Packard, 1999). Thus, insensitivity of eggs to variation in moisture levels may be an adaptation against extreme hydric conditions in lizard species that oviposit close to the soil surface. Although the causes are not yet clear, we conclude that eggs of different lizard species do not respond uniformly to variation in moisture levels (see also Ji & Braña, 1999).

PHENOTYPIC EFFECTS OF INCUBATION TEMPERATURE

Temperatures experienced by hatchlings during incubation profoundly modified several phenotypic traits. In most cases, these results were consistent with the phenotypic effects of qualitatively similar thermal incubation conditions used in our previous work on *B. duperreyi* (e.g. Shine, 1995; Shine & Harlow, 1996; Elphick & Shine, 1998).

We found strong effects of incubation temperature on hatching success, incubation period, tail length and antipredator behaviour. First, eggs incubated in warm conditions were 1.3 times more likely to hatch than cool-incubated eggs, suggesting that among-nest variation in temperatures may be an important determinant of egg mortality. Second, warm-incubated hatchlings had a 21% shorter incubation period than cool-incubated hatchlings (cf. Shine, 1995; Shine & Harlow, 1996; Elphick & Shine, 1998). Incubation period is likely to affect fitness, because *B. duperreyi* lives close to the upper elevational limit for oviparous reptiles in Australia. Long incubation periods experienced by cool-incubated hatchlings may result in eggs hatching after the onset of harsh winter conditions (Elphick & Shine, 1998). Third, warm-incubated hatchlings had 9.5% longer tails than cool-incubated lizards (cf. Shine, 1995; Elphick & Shine, 1998). Although evidence suggests that offspring size can be an important component of fitness in reptiles (Ferguson & Fox, 1984), it is unclear whether longer-tailed *B. duperreyi* may have a survival advantage over individuals with shorter tails. However, variation in tail length may determine a lizard's ability to escape predators by tail autotomy (Congdon, Vitt & King, 1974). Alternatively, incubation-induced changes in tail length may be less costly in terms of fitness than changes in SVL: SVL is often positively correlated with body mass and clutch size. If thermal variation inevitably leads to variation in total body length, changes in tail length may be paid off by reduced variation in the presumably more important component of fitness, SVL. Fourth, cool-incubated lizards displayed antipredator behaviour 12 times more often than warm-incubated lizards (cf. Shine, 1995). The adaptive significance of the behavioural displays observed in hatchling *B. duperreyi* remains uncertain, but evidence from several lizard species suggests that they serve to redirect a predator's attack from the lizard's body to the tail (Dial, Weldon & Curtis, 1989).

Additionally, we found that for a given SVL temperature and moisture significantly interacted to affect a hatchling's body mass: cool–dry incubated lizards were slightly heavier than both cool–wet and warm–dry incubated lizards. At present, we do not have a clear physiological explanation for these incubation-induced patterns of body mass. However, given the small and non-significant contrasts, the biological significance of this effect remains questionable.

In summary, incubation temperature clearly affects several fitness-related hatchling traits in *B. duperreyi*. However, differences in body size, body shape and antipredator behaviour between warm- and cool-incubated *B. duperreyi* have been reported to decrease during ontogeny (Elphick & Shine, 1998). Thus, the evolutionary and ecological significance of the phenotypic effects of incubation temperature clearly deserves further study (see also Qualls & Shine, 1998, 2000).

PHENOTYPIC EFFECTS OF SEX

There is ample evidence for sexual dimorphism in hatchling traits of reptiles (e.g. Kopstein, 1941; Clark, 1963; Shine, 1993). For instance, Janzen (1995) found

sex-specific variation in plastron length among hatchlings of the common snapping turtle (*Chelydra serpentina*), but the differences were small and probably not biologically significant. Similarly, it is not clear whether the minor sex differences in SVL, tail length and the relationship between a hatchling's body mass (and sprint speed) and its SVL detected in our experiment persist through ontogeny and contribute differently to fitness in male and female *B. duperreyi*.

More specifically, sex differences in phenotypic plasticity have rarely been documented by evolutionary biologists (but see Barker & Krebs, 1995 in *Drosophila aldrichi* and *D. buzzati*; Karavan *et al.*, 2000 in *D. melanogaster*), and incubation-induced sex differences in hatchling traits in particular have not received much attention. However, sex-specific phenotypic responses to incubation temperature have been found in hatchling reptiles, for instance affecting egg mortality in snakes (Burger & Zappalorti, 1988) and survival rates of hatchling turtles (Janzen, 1995). Recently, Elphick & Shine (1999) found that hatchling size and locomotor performance in *B. duperreyi* are differently affected by temperature in males compared with females, suggesting that the sexes differ in their plastic responses to thermal regimes experienced during embryogenesis. However, we did not find such temperature by sex interactions, presumably because we used a different thermal regime (present study, cool: 13 to 23°C, warm: 17 to 27°C; Elphick & Shine, 1999, cool: 16 to 24°C, warm: 23 to 31°C). Thus, the possibility remains that the expression of such interaction effects is strongly context-dependent. In our experiment, moisture differently affected incubation period in males and females, indicating that the two sexes differ in their norm of reaction for this trait. Although previous studies have shown that moisture levels can affect incubation periods in lizards (e.g. Packard *et al.*, 1980; Phillips & Packard, 1994), to our knowledge this is the first report of a moisture by sex effect on a hatchling lizard's trait. However, this effect was only minor, suggesting that it may not be biologically significant. Clearly, further studies are needed to assess how robust and context-dependent such patterns are.

Given the scarcity of data on sex by incubation environment effects, the ecological and evolutionary significance of such complex interactions remains unclear (Elphick & Shine, 1999). However, the future study of such interactions may yield promising insights into the evolution of environmental sex determination. More generally, sex differences in reaction norms are not well investigated and deserve further study.

CONCLUSIONS

In conclusion, lizards responded plastically to thermal incubation regimes whereas most traits were insensitive to moisture. As pointed out by Packard (1991), the failure of some authors to find phenotypic effects of moisture in hatchling lizards may be due to weaknesses in experimental design. However, in our study, it was unlikely that the lack of strong moisture effects resulted from the experimental design. Thus, we conclude that the contrasting results may reflect variation in phenotypic response among lizard species. This apparent variation might be due to differences in nesting behaviour among different species of lizard. If spatio-temporal variation in moisture conditions of nests of *B. duperreyi* is large and likely to be extreme, buffering against hydric perturbations may be a prime determinant of whether embryos complete development successfully. The hypothesis that developing eggs of many lizards ovipositing close to the soil surface are buffered against variation in hydric conditions is testable using comparative field and laboratory studies. In keeping with this idea, some of the clearest examples of moisture effects on hatchling lizards are found in species that oviposit in deep soil layers or construct nest cavities (Phillips & Packard, 1994). In contrast to many studies on turtles, this study does not support the generalization that water availability during embryogenesis is more important than temperature in determining the phenotypes of hatchling reptiles. Particularly, different lizard species are likely to differ in their phenotypic response to variation in substrate moisture levels.

ACKNOWLEDGEMENTS

We thank J. Bieri, M. J. Elphick, P. Harlow and J. Shoulder for logistic support, and C. G. Braendle, D. Ebert, M. J. Elphick, C. Haag, T. J. Kawecki, G. C. Packard, S. C. Stearns, M. B. Thompson, and D. Vizoso for comments on the manuscript. Comments by two anonymous referees greatly improved the presentation of our results. T. Flatt was supported by the Roche Research Foundation, the Dr Max Husmann Foundation, the Swiss Study Foundation, and the University of Basel (T.F.), and R. Shine was supported by the Australian Research Council.

REFERENCES

Ackerman RA. 1991. Physical factors affecting the water exchange of buried reptile eggs. In: Deeming DC, Ferguson MWJ, eds. *Egg incubation: its effects on embryonic development in birds and reptiles.* Cambridge: Cambridge University Press, 193–211.

Alberts AC, Perry AM, Lemm JM, Phillips JA. 1997. Effects of incubation temperature and water potential on growth and thermoregulatory behaviour of hatchling Cuban rock iguanas (*Cyclura nubila*). *Copeia* **1997:** 766–776.

Arnqvist G, Johansson F. 1998. Ontogenetic reaction

norms of predator-induced defensive morphology in dragonfly larvae. *Evolution* **79:** 1847–1858.

Barker JSF, Krebs RA. 1995. Genetic variation and plasticity of thorax length in *Drosophila aldrichi* and *D. buzzati*. *Journal of Evolutionary Biology* **8:** 689–709.

Burger J, Zappalorti RT. 1988. Effects of incubation temperature on sex ratios in pine snakes: differential vulnerability of males and females. *American Naturalist* **132:** 492–505.

Cagle KD, Packard GC, Miller K, Packard MJ. 1993. Effects of the microclimate in natural nests on development of embryonic painted turtles, *Chrysemys picta*. *Functional Ecology* **7:** 653–660.

Clark DRJ. 1963. Variation and sexual dimorphism in a brood of the western pygmy rattlesnake (*Sistrurus*). *Copeia* **1963:** 157–159.

Cogger HG. 1992. *Reptiles and amphibians of Australia* (Fourth edition). Sydney: Reed Books.

Congdon JD, Vitt LJ, King WW. 1974. Geckos: adaptive significance and energetics of tail autotomy. *Science (Washington)* **184:** 1379–1380.

Deeming DC, Ferguson MW. 1991. Physiological effects of incubation temperature on embryonic development in reptiles and birds. In: Deeming DC, Ferguson MWJ, eds. *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge: Cambridge University Press, 147–171.

Dial B, Weldon P, Curtis B. 1989. Chemosensory identification of snake predators (*Phyllorhynchus decurtatus*) by banded geckos (*Coleonyx variegatus*). *Journal of Herpetology* **23:** 224–229.

Donnellan SC. 1985. *The evolution of sex chromosomes in scincid lizards*. PhD thesis, Macquarie University, Sydney.

Elphick MJ, Shine R. 1998. Longterm effects of incubation temperature on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biological Journal of the Linnean Society* **63:** 429–447.

Elphick MJ, Shine R. 1999. Sex differences in optimal incubation temperatures in a scincid lizard. *Oecologia* **118:** 431–437.

Ferguson GW, Fox SF. 1984. Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* **38:** 342–349.

Goodman LA. 1971. The analysis of multidimensional contingency tables: stepwise procedures and direct estimation methods for building models for multiple classifications. *Technometrics* **13:** 33–61.

Gotthard K, Nylin S. 1995. Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. *Oikos* **74:** 3–17.

Greer AE. 1982. A new species of *Leiolopisma* (Lacertilia: Scincidae) from western Australia, with notes on the biology and relationships of other Australian species. *Records of the Australian Museum* **34:** 549–573.

Hamblin AP. 1981. Filter-paper method for routine measurement of field water potential. *Journal of Hydrology* **53:** 355–360.

Hurlbert SJ. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54:** 187–211.

Janzen FJ. 1995. Experimental evidence for the evolutionary significance of temperature-dependent sex determination. *Evolution* **49:** 864–873.

Ji X, Braña F. 1999. The influence of thermal and hydric environments on embryonic use of energy and nutrients, and hatchling traits, in the wall lizards (*Podarcis muralis*). *Comparative Biochemistry and Physiology A* **124:** 205–213.

Karavan D, Morin J-P, Gibert P, Moreteau B, Scheiner SM, David JR. 2000. The genetics of phenotypic plasticity. IX. Genetic architecture, temperature, and sex differences in *Drosophila melanogaster*. *Evolution* **54:** 1035–1040.

Kopstein F. 1941. Sexual dimorphism in Malaysian snakes. *Temminckia* **6:** 101–185.

Lee SK. 1978. An example for teaching some basic concepts in multidimensional contingency table analysis. *The American Statistician* **32:** 69–71.

Lindström J. 1999. Early development and fitness in birds and mammals. *Trends in Ecology and Evolution* **14:** 343–348.

Muth A. 1980. Physiological ecology of desert iguana (*Dipsosaurus dorsalis*) eggs: temperature and water relations. *Ecology* **61:** 1335–1343.

Overall K. 1994. Lizard egg environments. In: Vitt LJ, Pianka ER, eds. *Lizard ecology: historical and experimental perspectives*. Princeton: Princeton University Press, 51–72.

Packard GC. 1991. Physiological and ecological importance of water to embryos of oviparous reptiles. In: Deeming DC, Ferguson MWJ, eds. *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge: Cambridge University Press, 213–228.

Packard GC. 1999. Water relations of chelonian eggs and embryos: is wetter better? *American Zoologist* **39:** 289–303.

Packard GC, Packard MJ. 1988. The physiological ecology of reptilian eggs and embryos. In: Gans C, Huey RB, eds. *Biology of the reptilia*, Volume 16. New York: Alan Liss, 525–605.

Packard MJ, Packard GC, Boardman TJ. 1980. Water balance of the eggs of a desert lizard (*Callisaurus draconoides*). *Canadian Journal of Zoology* **58:** 2051–2058.

Packard GC, Packard MJ, Miller K, Boardman TJ. 1987. Influence of moisture, temperature, and substrate on snapping turtle eggs and embryos. *Ecology* **68:** 983–993.

Pengilley R. 1972. *Systematic relationships and ecology of some lygosomine lizards from southeastern Australia*. PhD thesis, Australian National University, Canberra.

Phillips JA, Packard GC. 1994. Influence of temperature and moisture on eggs and embryos of the white-throated savanna monitor *Varanus albigularis*: implications for conservation. *Biological Conservation* **69:** 131–136.

Qualls FJ, Shine R. 1998. Geographic variation in lizard phenotypes: the importance of incubation temperatures. *Biological Journal of the Linnean Society* **64:** 477–491.

Qualls FJ, Shine R. 2000. Post-hatching environment contributes greatly to phenotypic variation between two popu-

lations of the Australian garden skink, *Lampropholis guichenoti*. *Biological Journal of the Linnean Society* **71:** 315–341.

SAS Institute. 1989. *SAS/STAT User's Guide: Basics*, Version 6.04. Cary: SAS Institute Inc.

Shaw RG, Mitchell-Olds T. 1993. ANOVA for unbalanced data: an overview. *Ecology* **74:** 1638–1645.

Shine R. 1983. Reptilian viviparity in cold climates: testing the assumptions of an evolutionary hypothesis. *Oecologia* **57:** 397–405.

Shine R. 1993. Sexual dimorphism in snakes. In: Seigel RA, Collins JT, eds. *Snakes: Ecology and Behavior*. New York: McGraw-Hill, 49–86.

Shine R. 1995. A new hypothesis for the evolution of viviparity in reptiles. *American Naturalist* **145:** 809–823.

Shine R. 1999. Egg-laying reptiles in cold climates: determinants and consequences of nest temperatures in montane lizards. *Journal of Evolutionary Biology* **12:** 918–926.

Shine R, Elphick MJ, Harlow PS. 1995. Sisters like it hot. *Nature (London)* **378:** 451–452.

Shine R, Elphick MJ, Harlow PS. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* **78:** 2559–2568.

Shine R, Harlow PS. 1996. Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* **77:** 1808–1817.

Sokal RF, Rohlf FJ. 1995. *Biometry*, Third edition. New York: W.H. Freeman.

Sultan S. 1987. Evolutionary implications of phenotypic plasticity in plants. *Evolutionary Biology* **21:** 127–178.

Tracy CR. 1980. Water relations of parchment-shelled lizard (*Sceloporus undulatus*) eggs. *Copeia* **1980:** 478–482.

Travis J. 1994. Evaluating the adaptive role of morphological plasticity. In: Wainwright PC, Reilly PC, eds. *Ecological morphology: integrative organismal biology*. Chicago: University of Chicago Press, 99–139.

Van Damme R, Bauwens D, Braña F, Verheyen RF. 1992. Incubation temperature differentially affects hatching time, egg survival and sprint speed in the lizard *Podarcis muralis*. *Herpetologica* **48:** 220–228.

Via S, Gomulkiewicz R, De Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH. 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology and Evolution* **10:** 212–217.

Weisrock DW, Janzen FJ. 1999. Thermal and fitness-related consequences of nest location in painted turtles (*Chrysemys picta*). *Functional Ecology* **13:** 94–101.

Wilkinson L. 1989. *SYSTAT: The System for Statistics*. Evanston, IL: SYSTAT Inc.