

# Corticosterone Exposure during Embryonic Development Affects Offspring Growth and Sex Ratios in Opposing Directions in Two Lizard Species with Environmental Sex Determination

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## ABSTRACT

Stress experienced by a reproducing female can substantially affect the morphology, behavior, and physiology (and hence fitness) of her offspring. In addition, recent studies demonstrate that stress hormones (corticosterone) influence sex determination of embryos. To explore these issues, we manipulated corticosterone levels in eggs of two Australian lizard species (*Amphibolurus muricatus* and *Bassiana duperreyi*) that exhibit temperature-dependent sex determination (TSD). Elevated corticosterone levels during embryonic development affected body size, growth rates, and sex ratios of the resultant offspring, but the direction and magnitude of these effects differed between the species. Corticosterone enhanced growth rates of hatchling *B. duperreyi* but inhibited growth of *A. muricatus*. Eggs with elevated levels of corticosterone produced more daughters in *A. muricatus* and more sons in *B. duperreyi*. The sex-ratio effect in *A. muricatus* may have been due to sex-specific embryonic mortality, but it may represent a direct effect on sex determination in *B. duperreyi* (because embryonic mortality was not affected by corticosterone manipulation in this species). These results demonstrate the complexity of proximate mechanisms for sex determination among reptiles with TSD and illustrate the potential role of corticosterone in sex-determining systems.

## Introduction

Individual responses to environmental stressors depend on a variety of factors. For example, the approach of a predator

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induces flight in many potential prey species, but that response can be modified by features of the habitat (Templeton and Shriner 2004) or by the individual's reproductive status (Shine et al. 2003). Similarly, an intruding conspecific may induce vigorous territorial defense under some circumstances (Greenfield 1994; Hyman 2003; Peters and Ord 2003; Langkilde and Shine 2004) but dispersal in others (Enfjäll and Leimar 2005). Despite this complexity, many behavioral responses to stress tend to be associated with a common change in physiology, namely, an elevated production of glucocorticoids (corticosterone or cortisol) by the adrenal cortex (Harvey et al. 1984; Greenberg and Wingfield 1987). Because such physiological changes can directly affect individual survival and reproduction (Herrenkohl 1979; Romero and Wikelski 2001; Wingfield and Sapolsky 2003; Cote et al. 2006), we expect responses to elevated levels of corticosterone to be shaped by natural selection.

An extensive literature shows that elevated levels of stress, and hence circulating corticosterone, in reproductive females have long-lasting effects on offspring morphology, behavior, and physiology (McCormick 1999; Hayward and Wingfield 2004; Kapoor and Matthews 2005; Love et al. 2005; Meylan and Clobert 2005). For example, in rodents, maternally derived corticosterone is transferred to the developing fetus across the placenta (Zarrow et al. 1970) and can have long-term effects on physiological functions of offspring well into adulthood (Barbazanges et al. 1996; Kapoor and Matthews 2005). Similarly, in oviparous vertebrates (e.g., birds and reptiles), maternal corticosterone can be transferred to the egg yolk and affect hatchling growth, adult phenotype, and even reproductive success (Painter et al. 2002; Lovern and Wade 2003a; Hayward and Wingfield 2004; Naguib et al. 2006). Elevated corticosterone levels in developing lizard embryos can affect locomotor and antipredator behaviors of the hatchlings (Meylan et al. 2002; Meylan and Clobert 2004; Uller and Olsson 2006). Such maternal effects could be adaptive if prenatal exposure to corticosterone influences offspring phenotypes in ways that enhance survival and reproductive success in their respective environments. However, empirical support for the adaptive nature of these hormonally based maternal effects is scarce.

Corticosterone also may play a role in sex-determining pathways during development, particularly in birds (Pike and Petrie 2003, 2005b, 2006). For example, levels of maternally derived corticosterone in egg yolk often correlate with offspring sex (Pike and Petrie 2005b). Experimental manipulation of circulating corticosterone levels in reproductive quails suggests that high levels of this hormone can bias offspring sex ratios toward females (Pike and Petrie 2006). Corticosterone levels

experienced during development also influence sons differently than daughters (McCormick et al. 1995; Bowman et al. 2004). In many species, rates of growth and survival are more sensitive to corticosterone in males than in females (Meylan and Clobert 2005; Cote et al. 2006; Hayward et al. 2006).

Research on this topic has focused on species with chromosomal sex determination (birds, mammals, and many reptiles). Few studies have evaluated the role of corticosterone in species with environmental sex determination (ESD). Instead, research on the hormonal basis of sex determination in species with ESD has focused on estradiol or testosterone. These studies often demonstrate conflicting results in how maternally derived yolk estradiol and testosterone influence offspring sex ratios, particularly in reptiles with temperature-dependent sex determination (TSD; Radder 2007). Sex steroid hormone levels in egg yolk are related to offspring sex ratios in some species (Janzen et al. 1998; Bowden et al. 2000) but not in others (Radder 2007; Radder et al. 2007; Warner et al. 2007). Similarly, the direction of correlations between steroid hormone levels and offspring sex sometimes differ among species (Janes et al. 2007). These conflicting results are intriguing because testosterone levels are often closely associated with corticosterone levels (Ketterson et al. 1991), and therefore significant associations with testosterone may be confounded with the effects of corticosterone. Indeed, we recently reported that levels of maternally derived yolk corticosterone, but not testosterone, were associated with offspring sex in a lizard with TSD (Warner et al. 2007). In light of these conflicting correlative results, experimental manipulation of corticosterone levels in egg yolks are needed to disentangle the effects of these steroid hormones on sex determination.

In this study, our primary objective was to evaluate the effect of elevated corticosterone levels during embryonic development on sex determination and offspring phenotypes in two oviparous lizard species with TSD. Rather than manipulating maternal corticosterone levels, as has been done in most studies of birds and lizards (De Fraipont et al. 2000; Pike and Petrie 2006), we injected corticosterone directly into the egg soon after oviposition. By elevating corticosterone levels in the egg rather than in the mother, we ensured that corticosterone was delivered directly into the embryonic environment before the period of sex determination and differentiation.

## Material and Methods

### Study Species

We studied two oviparous lizard species from phylogenetically distinct lineages. Our first study species, the jacky dragon (*Amphibolurus muricatus*), is an agamid lizard that inhabits coastal heathland habitat of southeastern Australia (Cogger 2000). Jacky dragons have a TSD whereby cool (23°–26°C) and warm (30°–33°C) incubation temperatures produce female offspring and intermediate temperatures (27°–29°C) produce approximately balanced sex ratios (Harlow and Taylor 2000; Warner and Shine 2005). Maternally derived corticosterone levels in egg yolks may influence sex determination ( $P = 0.06$ ; Warner

et al. 2007), but this observation has yet to be explored with properly controlled experiments.

Our second study species is a scincid lizard (*Bassiana duperreyi*) from montane regions of southeastern Australia. This species exhibits male chromosomal heterogamety, but low incubation temperatures override the sex chromosomes such that cool incubation produces highly male-biased sex ratios (Shine et al. 2002; Radder et al. 2008b). Sex determination in this species is also associated with egg size such that large eggs overproduce female offspring (Shine et al. 2002). Levels of maternally derived steroid hormones (testosterone and estradiol) within egg yolks are not associated with offspring sex in this species (Radder et al. 2007), but the role of corticosterone has not been evaluated. All procedures described below have been approved by the University of Sydney Animal Care and Ethics Committee (projects L04/7-2004/3/3885 and L04/10-2005/1/4219).

### Protocol for *Amphibolurus muricatus*

We obtained clutches from 18 females from a captive colony of *A. muricatus*; see Warner and Shine (2005) and Warner et al. (2007) for a detailed description of housing conditions. Within 24 h of oviposition, eggs were weighed and assigned to one of four treatments. Although jacky dragons produce multiple clutches in a single season, only eggs from the second clutch of the season were used in this experiment (hence, only one clutch per female was used). Eggs from each clutch were allocated among four treatments to evaluate the effect of yolk corticosterone on offspring sex, morphology, and performance. For the first treatment ( $n = 32$  eggs), we used a syringe (24-gauge needle) to deliver corticosterone (Sigma, C-2505) directly into the egg yolk (concentration of 5  $\mu\text{g}/5 \mu\text{L}$ ). The second treatment ( $n = 32$  eggs) was used as a vehicle control; only sesame oil (0.5 mL) was inserted into the egg. Eggs allocated to the third treatment ( $n = 15$ ) were punctured with a syringe needle, but no substance was inserted. Eggs allocated to the final treatment ( $n = 19$ ) were not manipulated in any way. All treatments were performed within 2 h after eggs were removed from their nest (i.e., within 24 h of oviposition).

Immediately after treatment, each egg was placed into an individual glass jar (120 mL) filled with moist vermiculite ( $-200$  kPa). Eggs were half buried in the vermiculite, and glass jars were covered with plastic kitchen wrap (sealed with a rubber band) to prevent evaporation. All eggs were placed in one of two incubators set at a constant 28°C, a temperature that produces mixed offspring sex ratios in this species (Harlow and Taylor 2000). Eggs were rotated among and within incubators 3 times/wk to remove any effects of minor thermal heterogeneity within and between incubators.

After eggs hatched, hatchling lizards were weighed, measured (snout-vent length, SVL; tail length, TL), and sexed by manual eversion of hemipenes (Harlow 1996). We also measured the jaw length, head width, and head depth of each hatchling as described by Harlow and Taylor (2000). Each hatchling was given a unique combination of toe clips for individual iden-

tification and then housed in groups of five in outdoor enclosures (30 cm high  $\times$  36 cm wide  $\times$  50 cm long) for approximately 1 mo. Enclosures contained branches for perching and basking and shelter for hiding. Hatchlings were fed crickets (dusted in vitamin/mineral mix) 3 times/wk, and water in small bowls was always available. On March 7, 2007, all hatchlings were measured (SVL and TL), weighed, and released in the field at the capture location of their mother. These final size measurements enabled us to evaluate offspring growth rate while in captivity.

#### *Protocol for Bassiana duperreyi*

Eggs of *B. duperreyi* were obtained from five natural nests in the field on November 29, 2006. Our frequent searches for nests under rocks ensured that eggs were collected within 1–2 d of oviposition. Because *B. duperreyi* oviposit communally (Radder and Shine 2007), clutches from several females were found within a single nest; hence, the maternal identity of each egg was unknown. Indeed, all nests contained  $>20$  eggs, and because *B. duperreyi* has a clutch size of 3–9 eggs, we were confident that eggs in a single nest were from multiple females. Thus, eggs from a single nest were randomly allocated to three treatments to evaluate the effect of corticosterone on offspring phenotype.

We used a similar experimental design as described above for *A. muricatus*. For the first treatment ( $n = 53$  eggs), we used a syringe (24-gauge needle) to deliver corticosterone directly into the egg yolk, using the same dosage described for *A. muricatus*. The second treatment ( $n = 39$  eggs) was used as a vehicle control; only sesame oil (0.5 mL) was injected into the eggs. Eggs allocated to the third treatment ( $n = 15$ ) were not manipulated in any way. A treatment in which eggs were simply punctured was not used because previous work demonstrates no effect of puncturing eggs on offspring phenotypes in *B. duperreyi* (Radder et al. 2007).

Immediately after treatments each egg was placed into an individual glass jar (64 mL) filled with moist vermiculite ( $-200$  kPa). Eggs were half buried in the vermiculite, and jars were covered with plastic kitchen wrap to prevent evaporation. All eggs were placed in a single incubator set at a mean of  $22^{\circ}\text{C}$  and fluctuating  $\pm 7.5^{\circ}\text{C}$  daily. This thermal regime produces balanced offspring sex ratios (Shine et al. 2002). Eggs were rotated within the incubator once/wk.

After eggs hatched, hatchling lizards were weighed, measured (SVL and TL), and sexed by manual eversion of hemipenes (Harlow 1996). Each hatchling was marked (toe clipped) and then housed individually in a small plastic box (7 cm high  $\times$  13 cm wide  $\times$  22 cm long) for approximately 1 mo. Plastic boxes were kept on a wooden rack in a temperature-controlled room ( $24^{\circ}\text{C}$ ) with a 12L : 12D photoperiod. An underfloor heating element provided a thermal gradient of  $20^{\circ}$ – $35^{\circ}\text{C}$  in each box for 8 h/d. Each plastic box contained a shelter site and a water dish. The hatchlings were fed crickets (dusted in vitamin/mineral mix) 3 times/wk, and water in small bowls was always available. After 1 mo, all hatchlings were measured

(SVL and TL), weighed, and released at the nest site where eggs were collected. These final size measurements enabled us to evaluate offspring growth rate in captivity.

For both study species, the dosage of corticosterone that we used produces levels within the physiological range of corticosterone levels in egg yolks; our dosages were standardized based on corticosterone concentrations found in natural egg yolks of both species (Radder et al. 2008a; Warner et al. 2008). One caveat, however, is that our manipulations occurred immediately after oviposition, a time when maternally induced increases in corticosterone levels do not normally occur. Nevertheless, this timing of manipulation is likely to have a similar effect on offspring as would natural (immediately preoviposition) deposition of hormones. In nature, transfer of hormones from the maternal circulation into the egg yolk is probably possible until the eggs are shelled. The process of shell deposition can take as little as 24 h, followed almost immediately by oviposition (Packard and DeMarco 1991). Because this interval between shelling and oviposition is a very small part of total embryonic development, our postoviposition manipulation is likely to have had similar effects to those that occur naturally.

#### *Measure of Locomotor Performance*

At approximately 2 wk of age, we measured the locomotor performance of each hatchling (in both species) by chasing individuals along an electronically timed racetrack. The racetrack (1 m long) contained five infrared photocells spaced at 25-cm intervals and connected to an electronic stopwatch. Before racing trials, hatchlings were given at least 30 min to acclimate to room temperature; *A. muricatus* hatchlings were raced at  $32^{\circ}\text{C}$ , near their preferred body temperature (Heatwole and Firth 1982), and *B. duperreyi* hatchlings were raced at  $25^{\circ}\text{C}$ . Hatchlings were placed at one end of the racetrack and encouraged to run by gently touching their tails with a paintbrush. Walls along the sides of the track prevented hatchlings from jumping out. We recorded each 25-cm-split time over the entire 1-m distance as well as the number of stops lizards made over the 1-m distance. Each hatchling was raced four times with at least 3 min rest between successive trials for *A. muricatus* and three times with at least 10 min rest between successive trials for *B. duperreyi*. The fastest speeds measured over the 25-cm interval and 1-m distance were used in our analyses of maximal sprint performance.

#### *Statistical Analyses*

All analyses were performed with SAS software, version 9.1 (SAS Institute 1997). Before analyses, all variables were checked for normality and were log-transformed when needed to meet the assumptions of parametric analysis. Data analyses differed slightly between data sets for each study species because of differences in the way data were collected and in experimental design. For *A. muricatus*, the puncture treatment was combined with the unmanipulated controls because preliminary analyses

Table 1: Effect of corticosterone treatments (injection into the freshly laid egg) and sex on hatchling phenotypes in two lizard species with temperature-dependent sex determination

Covariate	<i>Amphibolurus muricatus</i>						<i>Bassiana duperreyi</i>						
	Treatment		Sex		Interaction		Treatment		Sex		Interaction		
	F	df	F	df	F	df	F	df	F	df	F	df	
Trait:													
Incubation length (d)	...	.6	2, 33	.1	1, 33	.2	2, 33	2.5	1, 84	<b>4.6*</b>	<b>1, 84</b>	1.8	1, 84
SVL (mm)	Egg mass	3.2	2, 32	.8	1, 32	1.0	2, 32	<b>64.3***</b>	<b>1, 83</b>	<b>5.7*</b>	<b>1, 83</b>	.3	1, 83
Mass (g)	Egg mass	<b>4.6**</b>	<b>2, 32</b>	1.1	1, 32	.3	2, 32	3.9	1, 83	<b>1.7</b>	<b>1, 83</b>	1.8	1, 83
Tail length (mm)	SVL	2.4	2, 31	.1	1, 31	.2	2, 31	.1	1, 82	<b>6.4*</b>	<b>1, 82</b>	2.4	1, 82
Body condition (mass g)	SVL	1.5	2, 32	.9	1, 32	.1	2, 32	<b>4.5*</b>	<b>1, 83</b>	<b>9.0**</b>	<b>1, 83</b>	1.4	1, 83
Head measures:													
Jaw length (mm)	SVL	2.2	2, 32	.3	1, 32	.3	2, 32	...	...	...	...	...	...
Jaw width (mm)	SVL	.1	2, 32	2.1	1, 32	.5	2, 32	...	...	...	...	...	...
Head depth (mm)	SVL	.1	2, 32	.2	1, 32	.2	2, 32	...	...	...	...	...	...
Running speed:													
Over 1 m (m/s)	SVL	.9	2, 27	.0	1, 27	.4	2, 27	1.1	1, 80	<b>5.3*</b>	<b>1, 80</b>	.0	1, 80
Over 25 cm (m/s)	SVL	.9	2, 27	.9	1, 27	.7	2, 27	.5	1, 79	3.4	1, 79	1.5	1, 79
Number of stops over 1 m	...	1.0	2, 26	3.4	1, 26	1.2	2, 26	1.4	1, 81	1.0	1, 81	.5	1, 81
Growth rate:													
SVL ( $\Delta$ mm/d)	...	2.3	2, 31	.2	1, 31	.3	2, 31	<b>17.2***</b>	<b>1, 81</b>	.2	1, 81	.8	1, 81
Mass ( $\Delta$ mass/d)	...	<b>4.4*</b>	<b>2, 31</b>	.3	1, 31	.2	2, 31	2.2	1, 81	1.3	1, 81	2.5	1, 81

Note. Two-factor ANOVA or ANCOVA were used separately for each species. SVL = snout-vent length. Values in boldface type are significant.

\*  $P \leq 0.05$ .

\*\*  $P \leq 0.01$ .

\*\*\*  $P < 0.001$ .

demonstrated no effect of puncturing eggs on offspring sex ratios or phenotypes (all  $P > 0.05$ ). Hence, our analyses of the *A. muricatus* data set evaluated sex ratio and phenotypic difference among offspring from three experimental treatments (corticosterone treatment, vehicle control, and unmanipulated control). For *B. duperreyi*, however, because a smaller sample of eggs was allocated to the unmanipulated control treatment ( $n = 15$ ) relative to that in the other treatments ( $n = 53$  and 39 for the experimental and vehicle control treatments, respectively), we pooled data from both control treatments to balance sample sizes between the corticosterone and control treatments.

Treatment effects on egg survival were evaluated with logistic regression. Two-factor ANOVAs and ANCOVAs were used to evaluate the effects of treatment and offspring sex (independent variables) on incubation duration (i.e., days between oviposition and hatching) and offspring phenotypes. We used egg mass as a covariate in analyses of offspring body size (SVL and mass). Body condition was evaluated using body mass as the dependent variable and SVL as the covariate. SVL was used as a covariate for analyses of tail length and running speed. For *A. muricatus*, SVL was used as a covariate for analyses of head dimensions (head dimensions were not measured in *B. duperreyi*). Growth rate was calculated as the change in size (in both SVL and mass) between hatching and release divided by the number of days between measurements. Because maternal identity was known for the *A. muricatus* data set, we included this factor as a random effect in our models. All analyses for *A. muricatus* were based on clutch mean values within each treat-

ment by sex combination. Because maternal identity was unknown for *B. duperreyi*, analyses for this species were based on values for individual eggs. Treatment effects on offspring sex ratios were evaluated with logistic regression with a logit link function, using sex (binomial response variable) as the dependent variable and treatment as the independent variable. In a final analysis, we evaluated species-specific responses to elevated yolk-corticosterone levels using logistic regression with species and treatment and their interaction as independent variables and offspring sex as the dependent variable; data from the control treatments were pooled in this analysis such that comparisons were made between the corticosterone and control treatments for both species.

## Results

### Egg Incubation and Survival

Corticosterone application did not affect the duration of incubation for either species (Table 1). Mean incubation duration was 53.3 d (SD = 3.4) for *Amphibolurus muricatus* and 49.1 d (SD = 4.7) for *Bassiana duperreyi*. Corticosterone application, however, did influence hatching success in *A. muricatus* but not in *B. duperreyi*. In *A. muricatus*, eggs treated with corticosterone had a lower survival rate (46.9%) than did those from the vehicle (93.8%) and unmanipulated controls (88.2%; Wald  $\chi^2 = 18.8$ ,  $P < 0.001$ ). For *B. duperreyi*, corticosterone application decreased egg survival slightly but not significantly (corticosterone treatment = 73.6% survival; control treatment = 85.2% survival; Wald  $\chi^2 = 2.1$ ,  $P = 0.14$ ).

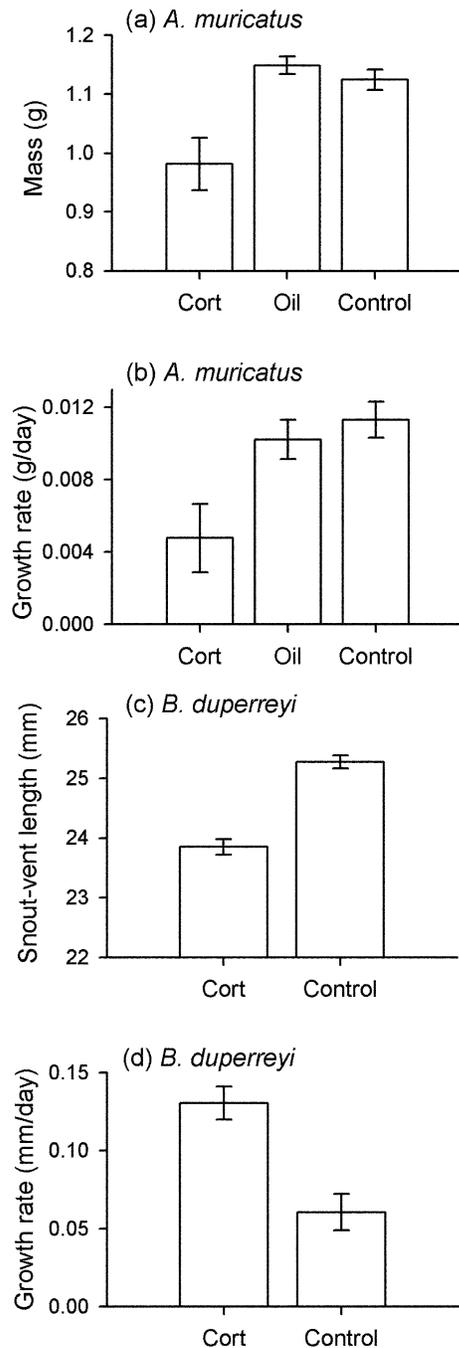


Figure 1. Effect of corticosterone application to eggs on offspring size and growth rate in two lizard species with temperature-dependent sex determination. For *A. muricatus*, analyses compared the corticosterone treatment (*Cort*), vehicle control (*Oil*), and control (*Control*; pooled data from punctured eggs and unmanipulated eggs). For *B. duperreyi*, analyses compared the corticosterone treatment (*Cort*) with the control treatments (*Control*; pooled data from vehicle control [oil] and unmanipulated eggs). *a*, Corticosterone effects on hatchling body mass (least squares means  $\pm 1$  SE) for *A. muricatus*. *b*, Corticosterone effects on hatchling growth rate in mass (mean  $\pm 1$  SE) for *A. muricatus*. *c*, Corticosterone effects on hatchling snout-vent length (SVL; least squares means  $\pm 1$  SE) for *B. duperreyi*. *d*, Corticosterone effects on hatchling growth rate in SVL (mean  $\pm 1$  SE) for *B. duperreyi*.

#### Hatchling Morphology and Performance

Corticosterone application to eggs influenced the size and growth rates of hatchlings for both species (Table 1). Hatchlings from corticosterone-treated eggs weighed less than those from the control treatment for *A. muricatus* and were shorter in SVL for *B. duperreyi* (Fig. 1). Corticosterone application to eggs enhanced hatchling body condition in *B. duperreyi* but not in *A. muricatus* (Table 1). Surprisingly, corticosterone had opposing effects on offspring growth rates in the two species; corticosterone application to eggs reduced growth rates of hatchling *A. muricatus* but enhanced growth rates of hatchling *B. duperreyi* (Fig. 1). Other phenotypic traits of offspring were not influenced by our manipulations. For *B. duperreyi* but not *A. muricatus*, offspring sex explained significant variation in offspring morphology and performance (Table 1). The effects of corticosterone on hatchling phenotypes did not differ between male and female offspring in either species (as indicated by nonsignificant treatment by sex interactions for all traits).

#### Hatchling Sex Ratios

Corticosterone had opposite effects on offspring sex ratios in *A. muricatus* and *B. duperreyi* (Fig. 2). Control treatments for both species produced approximately balanced sex ratios. In *A. muricatus*, corticosterone-treated eggs produced a female-

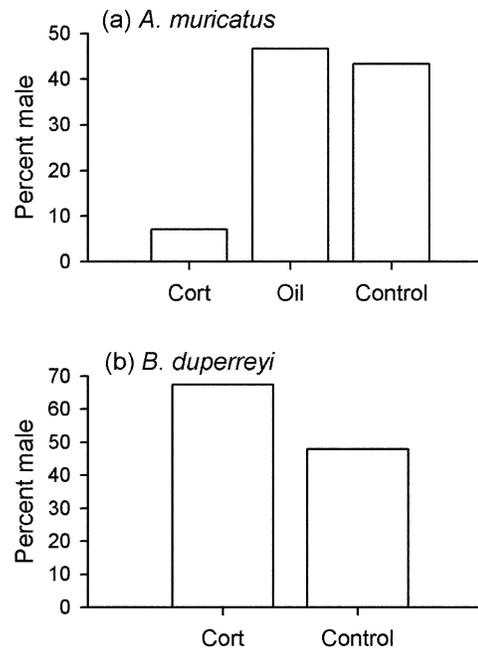


Figure 2. Effect of corticosterone application to eggs on offspring sex ratios in two lizard species with temperature-dependent sex determination. For *Amphibolurus muricatus*, analyses compared the corticosterone treatment (*Cort*), vehicle control (*Oil*), and control (*Control*; pooled data from punctured eggs and unmanipulated eggs). For *Bassiana duperreyi*, analyses compared the corticosterone treatment (*Cort*) with the control treatments (*Control*; pooled data from vehicle control [oil] and unmanipulated eggs). *a*, Offspring sex ratios of *A. muricatus*. *b*, Offspring sex ratios of *B. duperreyi*.

biased sex ratio (7.1% male; Wald  $\chi^2 = 4.7$ ,  $P = 0.030$ ). This pattern was reversed for *B. duperreyi*; although marginally non-significant, corticosterone-treated eggs produced a male-biased sex ratio (67.5% male; Wald  $\chi^2 = 3.3$ ,  $P = 0.069$ ). Accordingly, the interactive effect of species and corticosterone treatment on offspring sex ratios was significant (Wald  $\chi^2 = 6.9$ ,  $P = 0.008$ ).

## Discussion

Studies that evaluate the impact of prenatal steroid exposure on offspring phenotypes and sex ratios have produced conflicting results, and no general pattern across taxa has emerged (Petrie et al. 2001; Lovern and Wade 2003b; Daisley et al. 2005; Pike and Petrie 2005a; Saino et al. 2005; Radder 2007; Radder et al. 2007). Our study reinforces this conclusion: elevated steroid levels affected the embryos of two lizard species with TSD in very different ways.

Although we have no direct measures of offspring fitness, the magnitude of phenotypic effects induced by corticosterone manipulation suggests that fitness is likely to be impacted. However, such consequences may differ between species, with elevated yolk corticosterone increasing viability of *B. duperreyi* offspring but reducing that of *A. muricatus*. In *A. muricatus*, corticosterone application reduced both hatchling size and rates of posthatching growth. The former effect may have little impact on fitness (hatchling size does not predict subsequent survival; Warner and Shine 2007), but the latter effect may be important. In this species, rates of posthatching growth strongly influence body size and hence fitness in the following reproductive season (Warner and Shine 2005). Similar negative effects of corticosterone exposure occur in other organisms (Hayward and Wingfield 2004). In contrast, for *B. duperreyi*, any negative effect of corticosterone on hatchling size may be outweighed by the positive effect on growth that will likely enhance competitive ability later in life (Olsson 1992). Our results fit well with the conclusion from previous studies of lizards that prenatal corticosterone exposure may modify fitness-relevant behaviors (Meylan and Clobert 2005; Uller and Olsson 2006). However, because we did not measure behavioral phenotypes of offspring in this study, additional work is needed to understand the fitness consequences of prenatal exposure to elevated corticosterone levels.

The role of yolk steroid hormones in sex determination of ESD species is complex, and findings are often conflicting (Radder 2007). Although most previous work has focused on the role of estradiol and testosterone (Bowden et al. 2000; Elf 2003; Lovern and Wade 2003a), this study shows that corticosterone also affects offspring sex ratios and that its effect differs between species. Our data suggest that not only do the patterns differ, the pathways by which corticosterone affects offspring sex ratio may differ as well. In *A. muricatus*, the effect of corticosterone on offspring sex ratios is probably due to sex-specific embryonic mortality rather than to modified sex determination per se. If dead embryos from the corticosterone treatment are scored as males, the effect of corticosterone on offspring sex ratios dis-

appears (logistic regression:  $\chi^2 = 1.43$ ,  $P = 0.49$ ). This pattern suggests that corticosterone affects male embryos more negatively than female embryos. This contrasts with previous work on another lizard (*Lacerta vivipara*), in which corticosterone treatment enhanced survival of male hatchlings (Meylan and Clobert 2005). Female *A. muricatus* may enhance their fitness by allocating reproductive resources toward the production of daughters (rather than sons) when they are under stress. Previous correlative results on *A. muricatus* fit well with this notion: eggs with higher corticosterone levels tended to produce daughters (Warner et al. 2007).

The skewed sex ratios in *B. duperreyi* are more difficult to explain than those of *A. muricatus*. The low levels of embryonic mortality in both treatments suggest that corticosterone may play a direct role in sex determination. Indeed, a similar direct effect of corticosterone on sex determination has been demonstrated in birds (Pike and Petrie 2006), but this is the first demonstration of such an effect in a reptile. However, we still cannot rule out differential mortality of the sexes as a possible explanation, because even when dead embryos were scored as females, the effect became nonsignificant (logistic regression:  $\chi^2 = 0.049$ ,  $P = 0.82$ ). Nevertheless, the opposing direction of corticosterone effects on sex-specific embryonic mortality in *B. duperreyi* and *A. muricatus* is intriguing.

The opposing effects of corticosterone on offspring sex ratios may be associated with the mechanisms of sex determination exhibited by our study species. Although offspring sex is sensitive to incubation temperatures in both species and is labile soon after oviposition (Shine et al. 2007), *B. duperreyi* has heteromorphic sex chromosomes (Shine et al. 2002) whereas *A. muricatus* does not (Witten 1983). Thus, in *B. duperreyi*, the effect of corticosterone (as well as that of incubation temperature) needs to be strong enough to counteract the genetic effect on sex determination. Higher dosages of corticosterone than those employed in this study may further skew offspring sex ratios toward males. In *A. muricatus*, on the other hand, corticosterone probably exerted its sex-specific influence on embryo mortality after the initiation of sexual differentiation. Indeed, most eggs that died showed signs of death (i.e., fungal growth) 2–3 wk after oviposition, a time when sex determination has already occurred (when eggs are incubated at 28°C) in lizards with TSD (Bull 1987; Warner and Shine 2005; Shine et al. 2007). An additional concern is that there may be differences in the distribution of corticosterone in the egg yolk after injection (Groothuis and Von Engelhardt 2005). That is, if corticosterone does not dissolve in the yolk, unusually high doses could be taken in over a short period, thereby enhancing embryonic mortality. However, if this was the cause of increased mortality in eggs of *A. muricatus*, then we would have expected mortality to be random with respect to embryonic sex and thus to have produced a balanced sex ratio for the corticosterone-treated eggs that survived. Instead, the observed sex-ratio skew suggests that high mortality in this treatment was mediated via differential embryonic mortality between the sexes.

Overall, we show that elevated corticosterone exposure during embryonic development can effect phenotypes of offspring

in ways likely to affect their fitness, and the direction and magnitude of such effects differ among species. Hence, general conclusions about the role of prenatal corticosterone may prove to be elusive. We also provide the first demonstration that corticosterone affects sex determination in reptiles with TSD and show that the pathways and directions of that influence differ between species. We will need additional work to evaluate species-specific interactive effects of corticosterone with other steroid hormones and maternal abilities to transfer hormones into egg yolks in order to understand the complex sex-determining systems of squamate reptiles.

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