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Offspring Sex Is Not Related to Maternal Allocation of Yolk Steroids in the Lizard *Bassiana duperreyi* (Scincidae)

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ABSTRACT

The eggs of birds and reptiles contain detectable levels of several steroid hormones, and experimental application of such steroids can reverse genetically determined sex of the offspring. However, any causal influence of maternally derived yolk steroids on sex determination in birds and reptiles remains controversial. We measured yolk hormones (dihydrotestosterone, testosterone, and 17β-estradiol) in newly laid eggs of the montane scincid lizard *Bassiana duperreyi*. This species is well suited to such an analysis because (1) offspring sex is influenced by incubation temperatures and egg size as well as by sex chromosomes, suggesting that yolk hormones might somehow be involved in the complex pathways of sex determination, and (2) experimental application of either estradiol or fadrozole to such eggs strongly influences offspring sex. We obtained yolk by biopsy, before incubating the eggs at a temperature that produces a 50 : 50 sex ratio. Yolk steroid levels varied over a threefold range between eggs from different clutches, but there were no significant differences in yolk steroids, or in relative composition of steroids, between eggs destined to become male versus female. Further, yolk steroid concentrations were not significantly related to egg size. Thus, yolk steroid hormones do not appear to play a critical role in sex determination for *B. duperreyi*.

Introduction

In most animal species, sex is one of the primary axes of morphological and behavioral variation within a population. Accordingly, the factors that determine an individual's sex have been debated for more than 3,000 years (Mittwoch 2000). Although genotypic sex determination (GSD) is the best-understood system, environmental conditions during development are known to bias offspring sex ratio in many insect, fish, amphibian, and reptile species (reviewed by Korpelainen [1990]). More surprisingly, recent data suggest that environmental sex determination (ESD) of this kind is compatible with GSD because numerous epigenetic factors can influence offspring sex even in species with GSD. For example, offspring sex ratio and/or sexual phenotype are influenced by food availability to reproducing females (in Seychelles warblers and zebra finches: Komdeur et al. 1997; Kilner 1998), the proximity of same- versus opposite-sex embryos in utero (in house mice and Mongolian gerbils: Clark et al. 1994; Vandenbergh and Huggett 1994), and the timing of insemination with respect to ovulation (in numerous mammals: James 1996; see Lovern and Wade 2003b for a review of such effects).

What factors interact with sex-specific genetic features to influence the sex of offspring? Maternal allocation of steroid hormones to eggs is a plausible factor because (1) eggs of birds and reptiles often contain significant amounts of maternally derived steroid hormones and (2) experimental application of excess hormone to eggshells of birds and reptiles can switch genetically determined sex (Wade et al. 1997; Freedberg et al. 2006). Reports of sex differences in yolk hormone levels suggest some kind of functional link between maternally derived yolk hormones and offspring sex: either hormones determine sex or mothers allocate different amounts of steroid hormones to eggs destined to carry male versus female embryos (see, e.g., Gil et al. 1999; Petrie et al. 2001; Lovern and Wade 2002, 2003a, 2003b; Correa et al. 2005; Pike and Petrie 2005). Such a sex-specific allocation seems feasible on physiological grounds because of widespread transfer of nutrients, gases, water, and steroid hormones from pregnant mothers to their developing embryos (Janzen et al. 1998, 2002; Rutstein et al. 2005). All steroid hormones are lipophilic and therefore highly yolk soluble, and they readily cross biological membranes. Thus, steroids can easily pass from mother into the yolk during vitellogenesis and females could potentially control allocation to each egg (Rutstein et al. 2005).

Previous research on sex-specific allocation of yolk steroids and their potential role in sex determination has been conducted primarily on birds; much less is known about the role of maternally contributed steroid hormones in the other major lineage of oviparous vertebrates (i.e., reptiles). Within the rep-
tiles, interest has focused on species of turtles, crocodilians, and lizards that exhibit a specific form of ESD whereby offspring sex is determined by the incubation temperatures experienced by eggs (temperature-dependent sex determination [TSD]). This work on reptiles has shown that yolk hormones are deposited during vitellogenesis and has suggested that relative levels of hormones in the yolk can influence the developing gonads (Elf 2004). As in birds, yolk is a source of both estradiol and testosterone in TSD turtles, alligators, and leopard geckos, and initial levels of both steroids are correlated with the sex of the hatchlings (Elf 2004). In contrast, little is known about the levels of yolk steroids, or the possibility of sex-specific allocation by mothers, in GSD species of reptiles. A recent study on the lizard Anolis carolinensis has suggested that mothers allocate yolk steroids relative to the destined sex of the egg (Lovern and Wade 2003b). Interestingly, however, recent studies dispute the validity of earlier reports of sex differences in yolk steroid concentrations between male and female eggs in birds (see, e.g., Eising et al. 2003; Pilz et al. 2005; Gil et al. 2006). These studies cast significant doubt on the general conclusion that reproducing females strategically modify yolk steroid levels relative to offspring sex and suggest instead that the observed correlations might reflect embryonically produced rather than maternally derived steroids. Clearly, there is a need for further observations on a wide variety of animals from different lineages and with different modes of sex determination.

The recent discovery of both GSD and TSD within a single reptile population (of the montane lizard Bassiana duperreyi [Gray 1838]; Shine et al. 2002) provides a unique opportunity to explore the possibility of differential maternal allocation of steroids into egg yolk and their possible sex-determining effects and potential interaction with genetic factors. Briefly, when eggs of B. duperreyi are incubated under relatively warm conditions (optimal incubation regimes), hatchling sex is determined by genetics: XX individuals develop as females and XY individuals as males (Shine et al. 2002). However, this system breaks down when eggs are incubated at the lower temperatures characteristic of high-elevation nests in the wild. Such an incubation regime produces sex ratios skewed toward males (i.e., some XX individuals develop as phenotypic males). Intriguingly, sex in B. duperreyi is also affected by a third factor; regardless of incubation temperature, larger eggs are more likely to produce female offspring (Shine et al. 2002). Finally, our laboratory experiments have shown that we can sex-reverse B. duperreyi by applying either the aromatase inhibitor fadrozole (to produce males) or estradiol (to produce females) to eggshells partly through incubation (R. Radder and R. Shine, unpublished data). Thus, we can use this unique study system to address the following questions: (1) Do eggs destined to become males versus females differ in their steroid profiles, as might be expected either if B. duperreyi mothers allocate yolk steroids differentially into genetically determined sons versus daughters or if steroids play a causal role in sex determination? (2) Is the amount or relative composition of sex steroids related to egg size, as might be expected if the effect of egg size on offspring sex is mediated by steroid allocation? and (3) Do yolk steroid levels vary primarily among eggs within a clutch or among clutches produced by different females (i.e., what is the magnitude of inter- vs. intraclutch variation)? Our study provides the first data on yolk steroid levels in eggs of any scincid lizard species.

Material and Methods

Study Species and Collection
Bassiana duperreyi are medium-sized (to 80 mm snout-vent length [SVL]) oviparous scincid lizards that are widely distributed through southeastern Australia. Extensive research on this species has focused on populations in the Brindabella Range 40 km west of Canberra (1,240 m a.s.l.; 148°50' E, 35°21' S) in the Australian Capital Territory (see, e.g., Shine et al. 1995, 1997, 2002; Shine and Harlow 1996; Flatt et al. 2001; Shine 2002, 2004). These cool, high-elevation sites are close to the upper elevational limits for oviparous reproduction by Australian lizards. Most female B. duperreyi nest communally during early summer (usually in the last week of November and the first two weeks of December), and individual clutches cannot be distinguished with confidence in the field. Each female produces a single clutch (3–11 eggs) during the annual breeding season. We collected gravid B. duperreyi from the Brindabella Range on November 24 and 29, 2004, and brought the animals to the University of Sydney. After they were weighed and measured, the females were housed in individual cages (each 22 cm × 13 cm × 7 cm) and allowed to oviposit. Each cage contained moist vermiculite, shelter items, and a water dish. The lizards were fed live crickets three times a week. The room was kept at an air temperature of 20°C and a 12L : 12D photoperiod. Heating was provided by means of an underfloor heating element to maintain a thermal gradient of 20°C–35°C within each cage for 8 h/d and falling to ambient room temperature overnight (see Shine and Harlow 1996 for details). The lizards had ample opportunity for behavioral thermoregulation. All cages were visually inspected twice every day.

Egg Incubation
As soon as eggs were found they were removed from the cages. All eggs were weighed and yolks sampled (for hormone assay) using a sterile syringe with a 24-gauge needle. Approximately 10% (25–30 mg) of yolk was removed from each egg (determined by reweighing eggs after yolk removal). The yolk was transferred into a 2-mL plastic Eppendorf tube and homogenized with 500 µL of distilled water with vigorous shaking and stored at −80°C until radioimmunoassay. After yolk removal, the eggs were placed individually in 64-mL jars containing moist (−200 kPa) vermiculite. The jars were kept in a cycling-
temperature incubator with a sinusoidal diurnal thermal cycle of 20° ± 7.5°C. This thermal regimes mimics conditions that eggs usually experience in the field and that produces sex ratios close to 50 : 50 (Shine 2002; Shine et al. 2002). Incubation under these conditions takes approximately 10–11 wk (Shine et al. 2002). Incubators were checked twice daily for hatchlings. Any hatchlings found were removed and sex was determined by squeezing the tailbase to manually evert hemipenes (Harlow 1996; Shine et al. 2002). This sexing method was confirmed by gonadal histology of 12 randomly selected samples at the age of 8–10 wk. Our hemipene sexing method was 100% congruent with gonadal histology.

Radioimmunoassay for Yolk Steroids

We used competitive-binding steroid radioimmunoassays (RIA) to measure levels of the three principal sex steroids: dihydrotestosterone (DHT), testosterone (T), and 17 β-estradiol (E$_2$). We followed the RIA procedure of Wingfield and Farner (1975) as modified by Schwabl (1993) for yolk steroid measurements. The yolk samples were made up to 1 mL using distilled water. To determine extraction recoveries, approximately 2,000 cpm of tritiated DHT, T, and E$_2$ (PerkinElmer, Victoria, Australia) was added to each sample to serve as tracer. After the samples were vortexed and allowed to equilibrate overnight at 4°C, the hormones were extracted using petroleum ether and diethyl ether (30 : 70 vol : vol). The hormones were reconstituted with 90% ethanol and allowed to stand overnight (−20°C) for sedimentation of neutral lipids, which were removed by centrifugation. The supernatant was dried and resuspended in (1 mL) 10% ethyl acetate in isooctane. The yolk extract was transferred onto a diatomaceous earth microcolumn consisting of a celite : ethylene glycol : propylene glycol upper phase and celite : water lower phase. The samples were directly applied to columns, and hormone separation was completed by eluting each fraction with a phase consisting of a celite : ethylene glycol : propylene glycol upper phase and celite : water lower phase. The samples were applied to a column consisting of a celite : ethylene glycol : propylene glycol upper phase and Celite : water lower phase. The samples were applied to a column consisting of a celite : ethylene glycol : propylene glycol upper phase and Celite : water lower phase.

Yolk samples were assayed in duplicate, and hormone concentrations were compared to a standard curve from 1 to 250 pg/mL for T and E$_2$ and 2 to 500 pg/mL for DHT. Steroid extraction recoveries averaged 54.16% for DHT, 56.88% for T, and 51.09% for E$_2$. The intra-assay variation, calculated as the coefficient of variation for standards, was 6.56% for DHT, 10.2% for T, and 4.9% for E$_2$.

Table 1: Amounts of the steroids dihydrotestosterone (DHT), testosterone (T), and 17 β-estradiol (E$_2$) in yolk from eggs of the lizard Bassiana duperreyi

<table>
<thead>
<tr>
<th>Hormone Added to Yolk (pg)</th>
<th>Hormone Measured in Yolk after Addition (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHT</td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>100</td>
<td>108 ± 3.5</td>
</tr>
<tr>
<td>250</td>
<td>268 ± 2.8</td>
</tr>
</tbody>
</table>

Note. Amounts are expressed as mean ± SE and were measured by radioimmunoassay after removing endogenous steroids by treatment with charcoal solution (10 mg/mL) and addition of known amount of exogenous steroids. n = 3 for each measurement. ND = not detectable.

Specificity and accuracy of the hormone assay were examined following the procedures of Schwabl (1993). From additional samples, we removed endogenous steroids by treatment of yolk with 1 mL of charcoal solution (10 mg/mL). We then added known amounts of exogenous steroids (0, 100, and 250 pg/mL of DHT, T, and E$_2$; from Sigma) to each sample. After equilibration for 24 h, steroids were extracted and subjected to radioimmunoassay. The measured steroid levels were similar to expected values indicating negligible interference arising from antibody/steroid interactions with egg yolk substances (Table 1).

Data Analyses

We calculated the sex ratio as the number of males divided by the total number of hatchlings (i.e., no. males/[no. males + no. females]; Wilson and Hardy 2002). We investigated possible sex differences in yolk steroid levels by two methods: initially using unpaired t-tests and later by Wilcoxon matched-pair signed-rank tests to compare males and females within each clutch. The latter method was adopted to avoid confounding effects that might arise because of among-clutch variation in yolk steroid levels (Elf 2004). For this purpose, we calculated clutch mean values for male and female eggs separately, and these mean values were used for data analyses. In some species, the sex of the developing embryo may be determined by ratios of T : E$_2$ or DHT : E$_2$ rather than by absolute amounts of any single hormone (Bogart 1987). Therefore, we calculated T : E$_2$ and DHT : E$_2$ ratios and analyzed them as described above. Relationships between maternal traits (SVL and body mass), egg size, and yolk steroid levels, and among different steroid levels within the egg, were elucidated by Pearson correlation coefficient analyses. Since we detected no significant relationships between maternal traits and steroid levels, among-clutch variation in yolk steroid levels was analyzed by one-way ANOVA. Significance level was accepted at P < 0.05. All data analyses were performed using Statview 5.0 software (SAS Institute, Cary, NC).
Results

Sex Differences in Yolk Steroid Levels

A total of 55 eggs from 12 clutches were used to obtain the data, yielding a sex ratio of 0.62 (n = 21 female and 34 male offspring), not significantly different from 50 : 50 (χ² = 1.57, df = 1, P = 0.21). At the time of oviposition, eggs destined to become male or female did not differ significantly in concentrations of any of the steroids that we assayed (Table 2; Fig. 1) or in the ratio measures that we calculated (Table 2).

The same pattern (or lack thereof) was evident from analyses based on mean values for male versus female eggs per clutch. Compared with their sisters, male offspring emerged from eggs with similar postlaying levels of yolk DHT (Table 2; Fig. 2a), T (Table 2; Fig. 2b), E₂ (Table 2; Fig. 2c), and ratios such as T : E₂ and DHT : E₂ (Table 2). Similarly, we did not detect significant interclutch variation in ratios such as T : E₂ (Table 3) and DHT : E₂ (Table 3).

Within- and Among-Clutch Variations in Yolk Steroid Levels

Clutches laid by different females varied significantly in terms of yolk T levels within eggs (Table 3) but not in DHT or E₂ levels (Table 3). Similarly, we did not detect significant interclutch variation in ratios such as T : E₂ (Table 3) and DHT : E₂ (Table 3). Neither maternal body length nor maternal body mass was significantly correlated with mean yolk steroid levels (maternal body length: r = 0.28, P = 0.39 for DHT, r = 0.43, P = 0.17 for T, and r = 0.42, P = 0.18 for E₂; maternal body mass: r = 0.14, P = 0.67 for DHT, r = −0.04, P = 0.90 for T, and r = 0.17, P = 0.60 for E₂).

In a comparison among clutches, a strong correlation was evident between mean levels of T and E₂ (r = 0.80, P = 0.0009) as well as between DHT and E₂ (r = 0.63, P = 0.026). These correlations mean that overall, some clutches displayed higher concentrations of all three steroids than did other clutches. However, egg mass was not significantly related to yolk steroid levels (r = −0.23, P = 0.09 for DHT, r = −0.21, P = 0.13 for T, and r = −0.17, P = 0.13 for E₂, n = 55 eggs) or to ratio measures such as DHT : E₂ (r = −0.21, P = 0.13, n = 55 eggs) or T : E₂ (r = −0.20, P = 0.13, n = 55 eggs).

Discussion

Eggs of Bassiana duperreyi contain measurable quantities of yolk steroids (T, DHT, and E₂) shortly after oviposition, with levels (in picograms per milligram) within the range reported previously for other reptile species (Elf 2003, 2004; Lovern and Wade 2003b). Eggs from some clutches contained much higher steroid levels than eggs from other clutches, but we found no clear links between steroid levels and offspring sex in comparisons either within or among clutches. Thus, females of this species apparently do not allocate steroids differentially to male and female offspring, and maternal allocation of yolk steroid hormones does not influence offspring sex in B. duperreyi.

Although early research produced conflicting results as to whether maternal steroids are transferred to embryos via egg yolk (Altmann and Hutton 1938; Hertelendy and Common 1965), more recent work using radioactively labeled steroids and steroid radioimmunoassays makes it clear that maternally derived steroids are common yolk constituents not only in birds (Arcos 1972; Elf and Fivizzani 2002) but also in fishes, turtles, alligators, and lizards (see, e.g., Conley et al. 1997; McCormick

![Figure 1. Sex steroid concentrations (mean ± SE) in yolks of newly laid eggs of the montane lizard Bassiana duperreyi compared to the sex of the hatching eventually emerging from that egg. The levels of sex steroids in freshly oviposited eggs did not differ significantly between eggs destined to produce males and females (unpaired t-test, t₀ = −0.51, P = 0.61 for dihydrotestosterone [DHT]; t₀ = −0.75, P = 0.46 for testosterone [T]; and t₀ = −0.69, P = 0.49 for 17β-estradiol [E₂]; n = 34 male eggs and 21 female eggs).](image)

Table 2: Statistical analyses of sex differences in levels of the yolk steroids dihydrotestosterone (DHT), testosterone (T), and 17β-estradiol (E₂) in eggs of the lizard Bassiana duperreyi

<table>
<thead>
<tr>
<th>Yolk Steroid</th>
<th>Unpaired t-Test (df = 53)</th>
<th>Wilcoxon Signed-Rank Test (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t Value</td>
<td>P</td>
</tr>
<tr>
<td>DHT</td>
<td>−.51</td>
<td>.61</td>
</tr>
<tr>
<td>T</td>
<td>−.75</td>
<td>.46</td>
</tr>
<tr>
<td>E₂</td>
<td>−.69</td>
<td>.49</td>
</tr>
<tr>
<td>DHT : E₂ ratio</td>
<td>−.32</td>
<td>.75</td>
</tr>
<tr>
<td>T : E₂ ratio</td>
<td>.02</td>
<td>.98</td>
</tr>
</tbody>
</table>

Note. The left columns of the table provide the results of unpaired t-tests comparing male and female offspring based on a total of 21 daughter-producing and 34 son-producing eggs. The right columns of the table provide the results from nonparametric (Wilcoxon signed-rank) tests on data on mean values for male and female offspring per clutch from 12 clutches. Regardless of which statistical test was used, there was no significant difference in yolk steroid levels between eggs producing male offspring and those producing female offspring.
Figure 2. Yolk steroid hormone levels (mean ± SE) of newly laid eggs in relation to hatchling sex and clutch identity in the lizard *Bassiana duperreyi* for (a) dihydrotestosterone (DHT), (b) testosterone (T), and (c) estradiol (E2). Overall, there was no sexual difference in yolk steroids between brothers and sisters within each clutch (Wilcoxon signed-rank test, for DHT; for T; for E2 levels). Numbers above the bars in a represent sample sizes for each sex in each clutch and remain the same for b and c.

Table 3: Among-clutch variation in levels of the yolk steroids dihydrotestosterone, testosterone, and 17β-estradiol in eggs of the lizard *Bassiana duperreyi*, based on data from 12 clutches

<table>
<thead>
<tr>
<th>Yolk Steroid</th>
<th>F Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrotestosterone (DHT)</td>
<td>1.63</td>
<td>.12</td>
</tr>
<tr>
<td>Testosterone (T)</td>
<td>2.83</td>
<td>.007*</td>
</tr>
<tr>
<td>17β-estradiol (E2)</td>
<td>1.33</td>
<td>.24</td>
</tr>
<tr>
<td>DHT: E2 ratio</td>
<td>.89</td>
<td>.55</td>
</tr>
<tr>
<td>T: E2 ratio</td>
<td>.95</td>
<td>.50</td>
</tr>
</tbody>
</table>

* df = 11, 43.

* Significant differences (P < 0.05).
Steroids were allocated by mothers into eggs destined to become female and male.

If maternally derived yolk hormones do not determine offspring sex and are not allocated in a sex-specific way, what is the biological role of such hormones? We can only speculate that sex steroids in the yolk may influence other aspects of offspring development, perhaps in ways that either minimize or enhance sibling competition (Schwabl et al. 1997; Lipar and Ketterson 2000; Eising et al. 2001; Navara et al. 2006). Alternatively, steroids may be present in the yolk simply as the by-product of hormonal regulation of maternal physiology because of passive uptake during yolk formation (see, e.g., Bowden et al. 2002; Janzen et al. 2002). This second (nonadaptive) explanation fits well with our observation that B. duperreyi clutches with higher levels of androgens (T and DHT) also had higher levels of E₂, perhaps reflecting simple variation among reproducing female lizards in overall hormone titers. We note that some bird species differentially allocate yolk steroids to male and female eggs, but sex steroids do not influence sex determination (Müller et al. 2002; Rutstein et al. 2005), so the functional roles (if any) of sex steroids in yolk remain unclear.

Our results provide a strong contrast to the only previous study of yolk hormones in a reptile with sex chromosomes. Lovern and Wade (2003b) demonstrated sex-specific allocation of yolk steroids in a lizard, the green anole A. carolinensis. However, their study species differs from ours (and indeed, from most other reptiles) in producing only a single egg at a time. The left and right ovaries are used in alternation, and ovulation occurs frequently (at an interval of 7–14 d; Smith et al. 1973; Andrews 1985). This unusual reproductive mode would allow reproducing female anoles to allocate different yolk hormones to different eggs. In contrast, female B. duperreyi ovulate multiple eggs from each ovary simultaneously and produce only a single clutch of eggs during the breeding season (Shine 2002). Unlike those of birds and A. carolinensis, all follicles for a given clutch in B. duperreyi (and most other reptiles) are yolked simultaneously and hence are exposed to the same maternal hormonal environment (Bowden et al. 2002). This mode of reproduction may constrain sex-specific maternal allocation of steroids because it would require a reproducing female to somehow produce a different quantity of androgens as well as estrogens for different eggs, without compromising her own reproductive physiology. Successful reproduction is a fine-tuned balance between various sex steroids and especially between androgens and estrogens (Whittier and Tokarz 1992).

Because sex steroid production is under the control of hypothalamic and pituitary hormones, mechanisms for selective local enhancement of specific hormone levels for differential sex steroid allocation are difficult to visualize. Thus, the lack of sex-specific yolk steroid allocation in B. duperreyi may reflect physiological constraints.

Recent studies have attempted to link interspecific variation in the T : E₂ ratio with modes of sex determination (see, e.g., Lovern and Wade 2003a). Birds and lizards with GSD have T : E₂ ratios >1, and experimental manipulations of yolk steroid levels in these species suggest that T may be more effective than E₂ in affecting early development (Schwabl 1996; Lipar and Ketterson 2000; Lovern and Wade 2003a). In contrast, T : E₂ ratios <1 have been reported in alligators and turtles with TSD, and experimental manipulations of yolk steroids suggest that E₂ plays an important role in sex determination (Wibbels et al. 1994; Bowden et al. 2000). Is the T : E₂ ratio indicative of functional differences in steroid roles in different clades or simply a reflection of phylogenetic history (Lovern and Wade 2003a)? Interestingly, B. duperreyi (which exhibits both GSD and TSD) has a T : E₂ ratio of 1.37 ± 0.18 (mean ± SE; i.e., >1, as in GSD birds and lizards), and our exogenous application of E₂ elicited a massive shift of sex ratios. These apparently unusual features of B. duperreyi may be related to its unusually complex multifactorial mode of sex determination.

Significant among-clutch variation in B. duperreyi for yolk T levels mirrors the results of earlier studies on alligators, several turtle species, and the leopard gecko (Elf 2004). Variation in yolk steroids among clutches might be the consequence of varying levels of circulating hormones present in different mothers during yolkling of the follicles in B. duperreyi, as suggested for other reptiles (Elf 2004). These differences among individual females in yolk hormone levels also might engender corresponding variation in growth rates of offspring independent of egg mass as in the case of other reptiles (Elf 2004) and birds (Navara et al. 2006). Several factors (such as maternal age, body size, diet, photoperiod, mating season, clutch interval, social status, etc.) may contribute to individual variation in levels of yolk hormones (Müller et al. 2002; Elf 2004; Rutstein et al. 2005). However, in this study we did not find any correlation between yolk androgen or estrogen levels in B. duperreyi and factors such as maternal body size, clutch size, or average egg mass. Similar observations were reported for two species of turtles, Chrysemys picta and Chelydra serpentina (Elf 2004). Further, more detailed studies are warranted to shed light on these aspects.

Overall, our study suggests that yolk steroids of maternal origin are not critical for sex determination in B. duperreyi. Androgen and estrogen concentrations were not correlated with either offspring sex or egg size. However, it remains possible that other yolk steroids (e.g., progesterone, corticosterone, etc.; Correa et al. 2005; Pike and Petrie 2005) play some role in sex determination, or that yolk androgens or estrogens are involved but in a more complex way, in combination with other factors (Pilz et al. 2005). Regardless, the multiple sex-determining systems within B. duperreyi make it an ideal model system with which to further investigate the mechanisms by which reproducing females can influence the sex of their offspring.
Acknowledgments

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Yolk Hormones in a Lizard

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