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Reproductive tradeoffs and yolk steroids in female leopard geckos, *Eublepharis macularius*

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Abstract

Life history theory predicts tradeoffs among reproductive traits, but the physiological mechanisms underlying such tradeoffs remain unclear. Here we examine reproductive tradeoffs and their association with yolk steroids in an oviparous lizard. Female leopard geckos lay two eggs in a clutch, produce multiple clutches in a breeding season, and reproduce for several years. We detected a significant tradeoff between egg size and the number of clutches laid by females during their first two breeding seasons. Total reproductive effort was strongly condition-dependent in the first season, but much less so in the second season. Although these and other tradeoffs were unmistakable, they were not associated with levels of androstenedione, oestradiol, or testosterone in egg yolk. Female condition and egg size, however, were inversely related to dihydrotestosterone (DHT) levels in egg yolk. Finally, steroid levels in egg yolk were not directly related to steroid levels in the maternal circulation when follicles were developing, indicating that steroid transfer to eggs is regulated. These findings suggest that maternal allocation of DHT could mitigate tradeoffs that lead to poor offspring quality (i.e. poor female condition) and small offspring size (i.e. small egg size).

Introduction

Life history theory predicts numerous tradeoffs among reproductive traits (Roff, 1992; Stearns, 1992). A classic example of such a tradeoff is between offspring size and number. Given that females have finite resources available for producing offspring, an increase in offspring size entails a decrease in offspring number. However, the nature of tradeoffs can depend on whether females use stored resources (i.e. capital), resources acquired during reproduction (i.e. income), or a combination of capital and income to produce offspring (Roff, 1992; Stearns, 1992; Bonnet *et al.*, 1998; Sakai & Harada, 2005). For instance, females that rely on capital to produce offspring are expected to display a positive relationship between body condition and total reproductive effort (number × size of offspring). In contrast, pure income breeders would not display this relationship; rather, their total reproductive effort should be proportional to resources accrued during reproduction. Individual variation in body condition for capital breeders or resource acquisition for income breeders can therefore obscure the expected tradeoff between offspring size and number. Other potentially confounding factors include allocation to reproduction vs. growth or current vs. future reproduction. To sum up, negative correlations between individual pairs of traits may be missed when multidimensional tradeoffs are ignored (Pease & Bull, 1988). These observations make it imperative to measure as many potentially competing traits as possible and to use multivariate statistics in their analysis.

Despite substantial theoretical and empirical work on reproductive tradeoffs, there has been relatively little effort to identify the physiological mechanisms that determine where an individual will fall along a proposed tradeoff (but see Sinervo & Svensson, 1998). In other words, what are the proximate signals that females use to

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allocate resources to one trait at the expense of another? The neuroendocrine control of reproduction is conserved in vertebrates, which facilitates the study of such signals and their connection to reproductive tradeoffs. Neurones in the hypothalamus produce gonadotropin-releasing hormone, which stimulates synthesis and secretion of follicle-stimulating hormone (FSH) by the anterior pituitary. In turn, FSH plays a key role in recruitment, growth, and maturation of ovarian follicles, as well as stimulating synthesis of 17β-oestradiol (E2) by growing follicles. In oviparous species, E2 stimulates synthesis of vitellogenin and very-low density lipoprotein (VLDLy) by the liver. Growing follicles sequester vitellogenin and VLDLy from the circulation in a process called yolking. Ovarian E2 also regulates development of the oviduct, which deposits albumin and manufactures the eggshell once eggs are ovulated and fertilized. Lizard and snake eggs, however, do not have a significant albumin layer so egg size in these groups is primarily determined during follicle development.

A straightforward prediction is that FSH and ovarian steroids should influence the tradeoff between clutch and egg size. Indeed, administration of exogenous FSH increased average clutch size from four to six eggs with a concomitant decrease in average egg size in side-blotched lizards (Sinervo & Licht, 1991a). Conversely, surgical ablation of yolking follicles decreased clutch size but increased egg size (Sinervo & Licht, 1991b). Changes in egg size in these studies were due to changes in yolk size. Taken together, these findings indicate that yolking follicles, whose number is determined by FSH levels, compete for a limited pool of vitellogenin and VLDLy. If clutch size were held constant one might expect linkage between circulating levels of E2 and egg size. Higher E2 levels would stimulate synthesis of more vitellogenin and VLDLy and the production of larger eggs. In this regard, it is interesting that maternally derived steroids are found in egg yolk and that these hormones can influence offspring phenotype in several avian species (Schwabl, 1993, 1996a; Gil *et al.*, 1999; Eising *et al.*, 2001; reviewed in Groothuis & von Engelhardt, 2005). Steroids have also been found in egg yolk from oviparous reptiles, but their function in this group is unclear (reviewed in Elf, 2003).

Here, we investigate relationships among maternal steroids, yolk steroids and reproductive tradeoffs in female leopard geckos (*Eublepharis macularius*). Steroidogenic enzymes convert cholesterol into androgens and oestrogens in vertebrates (Strauss, 2004). Androstenedione (AE) is the major precursor for synthesis of testosterone (T) which can be converted into dihydrotestosterone (DHT) or the main oestrogen E2. Circulating levels of DHT, E2, and T increase during the follicular/vitellogenic phase of the reproductive cycle in female leopard geckos (Rhen *et al.*, 2000) and are found in different quantities in egg yolks from different females. An unresolved question is whether maternal steroids passively diffuse into developing eggs or if steroid levels in eggs are actively regulated. If the former hypothesis were true, one would predict a positive relationship between maternal steroid levels during the reproductive cycle and steroid levels in eggs produced during that cycle. If steroid deposition in eggs were regulated, steroid levels in the maternal circulation would be unrelated (or even inversely related) to yolk steroids. We test these hypotheses by reexamining data on circulating levels of maternal steroids from Rhen *et al.* (2000) and collecting new data on yolk steroids in eggs produced during that study. In a second study, we examine multivariate relationships among yolk steroids and reproductive tradeoffs in female leopard geckos during their first and second breeding seasons. We specifically determine whether maternal steroids deposited in egg yolk vary within and between breeding seasons and if levels of yolk steroids co-vary with female condition, number of clutches laid, or egg size.

Methods

Animals

Animals were cared for in accord with a protocol approved by the Institutional Animal Care and Use Committee at the University of Texas. Eggs from our leopard gecko colony were collected and candled for fertility. Fertile eggs were incubated in moist vermiculite (1 part water : 1 part vermiculite) at one of four temperatures: incubation at 26 °C produces all females, 30 °C produces 75% females, 32.5 °C produces 25% females and 34 °C produces 95% females (Viets *et al.*, 1993). After hatching, geckos were raised in isolation as previously described (Flores *et al.*, 1994).

We have characterized the hormonal changes that occur during the reproductive cycle of female leopard geckos from each of the four temperatures just described (Rhen *et al.*, 2000). In brief, we collected blood samples and measured circulating levels of T, DHT and E2 when females were previtellogenic (i.e. had no visible follicles or eggs), when their follicles were <9 mm (mean ± SE = 7 ± 0.2 mm; $n = 33$) in diameter (i.e. during early vitellogenesis), when their follicles were 12–14 mm (mean ± SE = 13 ± 0.2 mm; $n = 33$) in diameter (i.e. during late vitellogenesis) and again 1–3 days after ovulation when females were gravid. Yolking follicles and oviducal eggs are clearly visible through the abdominal wall in reproductively active females. On average, it took females 35 days to produce a clutch of two eggs. Mothers of a given clutch were unambiguously identified because females are gaunt after oviposition and only one female in a cage laid eggs on a given day. Eggs were collected and frozen within 24 h of oviposition. Those eggs were used to assess the relationship between plasma and yolk steroid levels in the current study.

In the second study, nine sexually mature females (i.e. *c.* 1 year of age) from 30 °C were placed in one of two

breeding cages (61 cm wide, 61 cm deep, 45 cm high) with sexually experienced males from 32.5 °C. Five females were housed with one male and four females with a second male. Animals in breeding cages were fed vitamin and mineral coated mealworms *ad libitum* and had continuous access to water. Pinky mice were provided as supplemental food once a week. A propylene box (30 × 12 × 6 cm) provided shelter and was partially filled with moist sand for nesting. Female reproductive status was monitored weekly and nest boxes and cages checked daily for eggs. When eggs were found, we recorded the lay date and measured egg width and egg length to the nearest 0.1 mm and egg mass to the nearest 0.1 g. Eggs were placed in airtight vials labelled with a unique egg number, lay date and the parents' identification numbers. Eggs were frozen at −20 °C until yolk steroids were extracted and measured. Some eggs were lost so yolk steroids were not measured for all eggs in this study. We measured the mother's snout–vent length (SVL) to the nearest 0.1 cm and mass to the nearest 0.1 g immediately after eggs were discovered.

Radioimmunoassay

Frozen eggs were dissected and any embryos present were staged and preserved. Yolk was homogenized in phosphate-buffered saline (PBS) and an aliquot used for assay. Steroid hormones were extracted using a modified version of the method described by Schwabl (1993). Approximately 2500 CPM's of ^{3}H-AE, ^{3}H-DHT, ^{3}H-E2 and ^{3}H-T were added to homogenates to monitor recovery efficiencies and samples were allowed to equilibrate overnight at 4 °C. Hormone analysis was done as previously described (Elf *et al.*, 2002). Yolk extracts were applied to celite : propylene glycol : ethylene glycol (6 : 1.5 : 1.5, w : v : v) microcolumns with celite : water (3 : 1, w : v) glycol traps. The 2% ethyl acetate in isooctane fraction was eluted to collect AE, the 10% ethyl acetate in isooctane fraction was eluted to collect DHT, and the T and E2 were eluted with 20% and 40% ethyl acetate in isooctane respectively. Eluted fractions were dried under nitrogen at 40 °C and re-suspended in 1% gelatine in PBS and allowed to equilibrate overnight at 4 °C. AE was assayed from duplicate 50 μL aliquots, DHT was assayed from duplicate 100 μL aliquots, E2 was measured from duplicate 200 μL aliquots, and T was assayed from duplicate 50 μL aliquots, all using commercial ^{125}I-labelled RIA kits from Diagnostics Systems Laboratories (Webster, TX, USA). Free and bound hormones were separated by the addition of dextran-coated charcoal, a 15-min incubation, and centrifugation at 3000 ***g*** for 15 min at 4 °C. A 500 μL aliquot was counted using a Beckman 5500 gamma counter. Recovery efficiencies were 35.97%, 24.2%, 42.35% and 49.99% and assay sensitivities were 0.5 pg per assay tube, 0.2 pg per assay tube, 0.0625 pg per assay tube and 0.05 pg per assay tube for AE, DHT, E2 and T respectively. Aliquots from a pooled reserve of yolk were assayed in parallel with each set of samples to determine inter- and intra-assay coefficients of variation (CV). Inter-assay CV were 12.07%, 8.71%, 9.18% and 5.85% whereas intra-assay CV were 10.83%, 4.22%, 4.68% and 2.32% for AE, DHT, E2 and T respectively.

Statistical analyses

We used stepwise regression to determine relationships between plasma steroid levels during the reproductive cycle (data from Rhen *et al.*, 2000) and the levels of steroids deposited in egg yolk during that cycle (new data). Given that three plasma steroids (DHT, E2 and T) were measured at four reproductive stages, we first tested for multicollinearity among these variables. Although there were significant correlations among circulating steroids, the degree of collinearity was not high as assessed by pairwise correlations and the smallest characteristic root in a principal components analysis.

In the second study, we used female identity (random effect), breeding season and clutch number as independent variables in a mixed model, restricted maximum likelihood (REML) ANOVA. Female SVL, female mass, egg size, egg shape and yolk steroids were dependent variables. The data were unbalanced (i.e. females did not produce the same number of clutches in both seasons) so we had to drop the three-way interaction from the model. We used egg mass, egg length and egg width in a principal components (PC) analysis. The first PC was used as a measure of egg size [PC1 = (0.67 × egg mass) + (0.47 × egg length) + (0.58 × egg width)] and explained 63.7% of the variation in egg characteristics. The second PC was a measure of egg shape [PC2 = (−0.07 × egg mass) + (0.81 × egg length) + (−0.58 × egg width)] and explained 27.9% of the variation in egg characteristics. Levels of AE, DHT, E2 and T in egg yolk were $\log_{10}$-transformed to meet model assumptions. Given significant main and/or interaction effects in REML ANOVA, we used the Dunn–Sidák method to correct for multiple comparisons. Nominal significance levels were calculated as $\alpha' = 1 - (1 - 0.05)^{1/k}$, where k is the number of comparisons for an experiment-wise $\alpha = 0.05$.

We used partial correlations to test for multidimensional tradeoffs within and between breeding seasons. We specifically examined correlations among (1) female condition at the start of each season (mass/SVL after first clutch), (2) number of clutches laid in that season and (3) a female's average egg size (PC1) during that season. Given that egg size decreased with clutch order, using average egg size for an entire breeding season could spuriously inflate correlations among traits. Consequently, we also examined correlations with egg size at the start of each season (i.e. average egg size for the first two clutches). We used partial correlations to test for tradeoffs between breeding seasons; i.e. correlations

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between (1) total reproductive effort in the first season, (2) female condition at the start of the second season, (3) the number of clutches laid in the second season and (4) average egg size in the second season. Finally, we examined correlations among these reproductive traits and yolk steroids. All statistics were done using JMP 5.0.1.2 software (SAS Institute Inc., 2002).

Results

Relationship between circulating steroids and yolk steroids

Stepwise multiple regression revealed that maternal steroid levels during the reproductive cycle were related to steroid levels in eggs produced during that cycle (Table 1). Females with higher levels of T during late vitellogenesis laid eggs with higher levels of T (Table 1; Fig. 1a). Circulating levels of DHT after ovulation were also related to T levels in egg yolk (Table 1; Fig. 1b). In contrast, there was an inverse relationship between circulating levels of T after ovulation and T levels in egg yolk (Table 1; Fig. 1c). Levels of AE and DHT in egg yolk were also related to maternal steroids: regression coefficients were of the same sign and very similar in magnitude for maternal T during late vitellogenesis and maternal DHT and T after ovulation (Table 1). In contrast, the concentration of E2 in egg yolk was only related to maternal E2 levels after ovulation (Table 1). Gravid females with higher levels of E2 laid eggs with higher levels of yolk E2 (Fig. 1d).

Changes in female SVL, female mass and egg characteristics with clutch order and breeding season

Female SVL did not change across breeding seasons or with successive clutches (Table 2). In contrast, female mass changed across breeding seasons and with successive clutches (Table 2). There was also a significant season by clutch interaction for female mass (Table 2). Female mass decreased with successive clutches during the first season, but did not decrease as dramatically during the second season (Fig. 2a). There was a significant female by season interaction for mass (Table 2). Five females gained mass between the first and second seasons (change in mean values = +5.5, +6.0, +8.8, +9.2 and +12.8 g) whereas the other four females did not gain or lose significant mass between seasons (change in mean values = −3.5, +2.2, +2.7 and +5.2 g).

Egg size (PC1) changed across breeding seasons and with successive clutches (Table 2). On average, egg size increased from the first to the second season but decreased with clutch order within seasons (Fig. 2b). The decrease in egg size with successive clutches was similar in both seasons: i.e. there was no detectable interaction between season and clutch order (Table 2). In contrast to egg size, egg shape (PC2) did not differ between seasons or change with clutch order (Table 2).

Reproductive tradeoffs within and between breeding seasons

Bivariate and multivariate correlations revealed significant reproductive tradeoffs. The bivariate correlation between female condition (mass/SVL) at the start of the first season and the number of clutches laid in that season was significant, as was the correlation between female condition and average egg size (Table 3). In contrast, the bivariate correlation between number of clutches laid and egg size was not significant. Multivariate correlations were as predicted for a capital breeder (Table 3). Females in better condition laid more and larger eggs than females in poor condition. Controlling for female condition, there was a significant tradeoff between the number of clutches laid and egg size (i.e. a negative partial correlation). Multivariate correlations remained high even when average egg size for the first two clutches was used rather than average egg size for the entire season.

Table 1. Multiple regression analyses of androstenedione (AE), testosterone (T), dihydrotestosterone (DHT) and 17 β-oestradiol (E2) concentration in egg yolk as a function of maternal steroid levels ($n = 24$ for all analyses).

Dependent variable	Independent variables	Coefficient (SE)	t-Ratio	P-Value
Yolk T ($r^2 = 0.66$; $n = 24$))	Intercept	1.05 ± 0.53	1.96	0.06
	Maternal T (Late Vitellogenesis)	0.40 ± 0.13	3.07	0.006
	Maternal T (Gravid)	−1.25 ± 0.21	−6.02	<0.0001
	Maternal DHT (Gravid)	1.08 ± 0.24	4.48	0.0002
Yolk AE ($r^2 = 0.69$: $n = 23$)	Intercept	1.84 ± 0.60	3.08	0.006
	Maternal T (Late Vitellogenesis)	0.41 ± 0.14	2.87	0.01
	Maternal T (Gravid)	−1.47 ± 0.23	−6.39	<0.0001
	Maternal DHT (Gravid)	1.27 ± 0.27	4.68	0.0002
Yolk DHT ($r^2 = 0.53$; n=24)	Intercept	0.38 ± 0.53	0.70	0.49
	Maternal T (Late Vitellogenesis)	0.37 ± 0.13	2.8	0.01
	Maternal T (Gravid)	−0.92 ± 0.21	−4.41	0.0003
	Maternal DHT (Gravid)	0.86 ± 0.24	3.54	0.002
Yolk E2 ($r^2 = 0.42$; $n = 24$)	Intercept	1.65 ± 0.41	3.99	0.0006
	Maternal E2 (Gravid)	0.55 ± 0.14	4.02	0.0006

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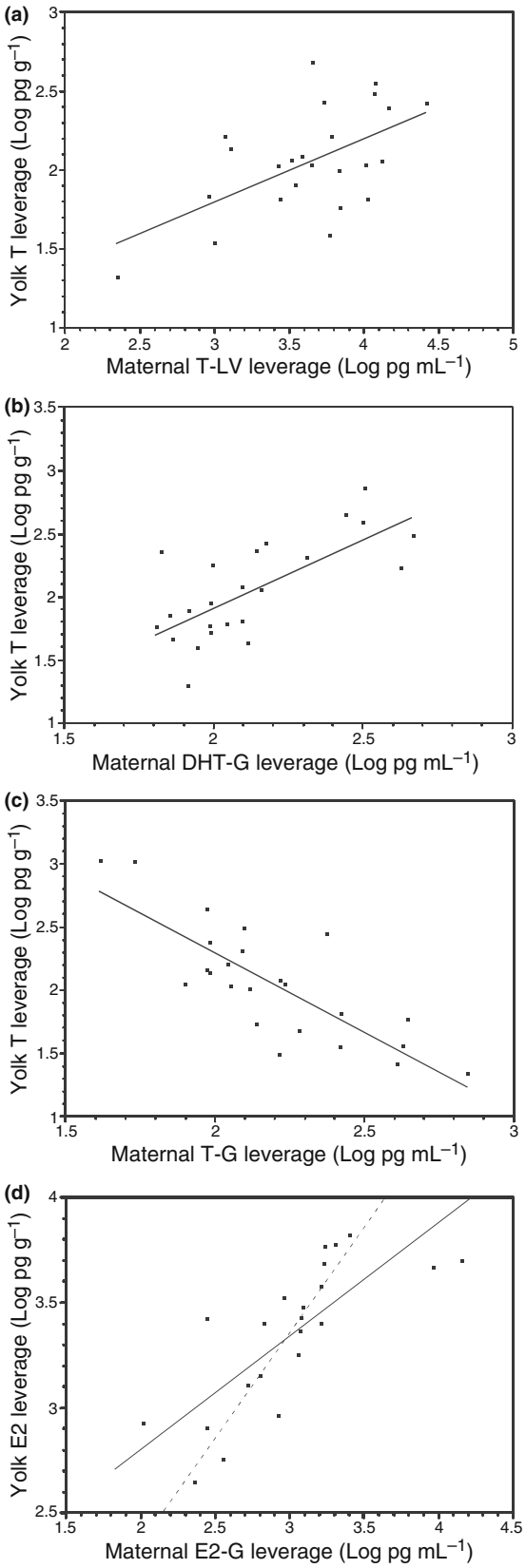

The pattern of tradeoffs changed from the first to the second season. This change was due to a decrease in variance in female condition, which was 15-fold greater at the start of the first season than it was at the start of the second season (Table 3). Females in poor condition at the start of the first season generally gained mass between seasons and were in better condition at the start of the second season. Females in good condition at the start of the first season were in similar condition at the start of the second season. In contrast, variance in egg size and the number of clutches laid did not change between seasons (Table 3). Consequently, the bivariate correlation between female condition and the number of clutches laid (or egg size) was not significant in the second season. However, the bivariate correlation between the number of clutches laid and egg size was negative and nearly significant. Multivariate correlations also reflect decreased variance in female condition (Table 3). Although the partial correlation between female condition and number of clutches laid (or egg size) was not significant, the tradeoff between number of clutches laid and egg size was significant. Partial correlations changed little when average egg size for the first two clutches was used rather than average egg size for the entire season (Table 3).

As female condition, number of clutches laid and egg size were so highly correlated in the first season, we used total reproductive effort (i.e. total egg mass) in the first season to test for tradeoffs between seasons. Reproductive effort in the first season did not influence female condition or egg size in the second season (Table 3). However, there was a significant tradeoff between reproductive effort in the first season and the number of clutches laid in the second season: females that invested more in reproduction in their first season produced fewer clutches in their second season (Table 3). Controlling for past reproductive effort, there was a significant partial correlation between female condition and the number of clutches produced during the second season (Table 3). The partial correlation between egg size and the number of clutches laid was consistent with previous estimates.

Changes in yolk steroids with clutch order and season

Overall, there were no consistent patterns of change in yolk steroids across breeding seasons or with successive clutches (Table 2; Fig. 3). Breeding season and clutch

Fig. 1 Steroid levels in the maternal circulation during the reproductive cycle predict steroid levels in eggs produced during that cycle. Leverage plots of yolk testosterone (T) concentration as a function of (a) maternal T concentration during late vitellogenesis, (b) maternal dihydrotestosterone (DHT) concentration after ovulation and (c) maternal T concentration after ovulation. (d) Leverage plots of yolk oestradiol (E2) concentration as a function of maternal E2 concentration after ovulation.

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Table 2. Restricted maximum likelihood ANOVA of female snout vent length, female mass, egg size, egg shape, yolk androstenedione, yolk dihydrotestosterone, yolk estradiol, and yolk testosterone (df = degrees of freedom; Var = variance component estimate; SS = sum of squares; F-ratio = F statistic; P = probability).

Source of variation	d.f.	Var	SS	*F*-value	*P*-value
Female SVL					
Female	8	0.074	1.61		
Season	1		0.07	0.89	0.37
Female × Season	8	0.033	0.84		
Clutch	4		0.63	2.07	0.11
Female × Clutch	30	0.001	0.04		
Season × Clutch	4		0.05	0.17	0.95
Error	9	0.076	0.68		
Female mass					
Female		5.4	22.8		
Season			36.0	9.5	0.015*
Female × Season		12.2*	226.2		
Clutch			306.1	20.1	<0.0001*
Female × Clutch		0.9	37.8		
Season × Clutch			82.6	5.4	0.017*
Error		3.8	34.2		
Egg size					
Female	8	0.27	3.0		
Season	1		3.8	10.0	0.014*
Female × Season	8	0.36	17.2		
Clutch	4		7.6	5.0	0.004*
Female × Clutch	28	0.25	14.9		
Season × Clutch	4		2.3	1.5	0.21
Error	63	0.38	24.0		
Egg shape					
Female	8	0.15	3.58		
Season	1		0.04	0.12	0.74
Female × Season	8	0.15	6.59		
Clutch	4		2.28	1.65	0.19
Female × Clutch	28	0.06	4.50		
Season × Clutch	4		0.49	0.36	0.84
Error	63	0.35	21.7		
Yolk AE					
Female	8	0.00	0.00		
Season	1		0.15	2.9	0.15
Female × Season	8	0.04	0.94		
Clutch	4		0.36	1.8	0.17
Female × Clutch	21	0.11*	5.17		
Season × Clutch	4		1.63	8.1	<0.0001*
Error	41	0.05	2.05		
Yolk DHT					
Female		0.008	0.095		
Season			0.003	0.12	0.75
Female × Season		0.006	0.084		
Clutch			0.229	2.26	0.10
Female × Clutch		0.033	1.394		
Season × Clutch			0.278	2.75	0.04*
Error		0.025	1.038		
Yolk E2					
Female	8	0.024	0.15		
Season	1		0.12	1.4	0.30
Female × Season	8	0.021	0.56		
Clutch	4		0.50	1.5	0.24
Female × Clutch	21	0.118	8.03		
Season × Clutch	4		0.29	0.9	0.49
Error	41	0.084	3.46		
Yolk T					
Female		0.000	0.001		
Season			0.015	0.81	0.41
Female × Season		0.002	0.031		
Clutch			0.070	0.97	0.44
Female × Clutch		0.031	1.784		
Season × Clutch			0.257	3.57	0.014*
Error		0.018	0.737		

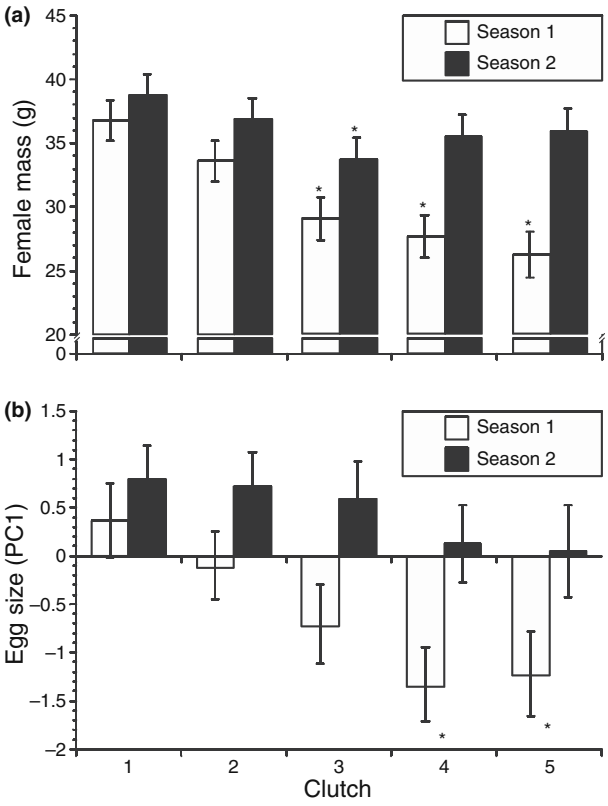

Fig. 2 (a) Female mass after laying the indicated number of clutches in the first and second breeding seasons. (b) Relative egg size as a function of clutch number and breeding season. Egg size is the first principal component of egg measurements as described in the text. Female mass and egg size are least squares means (±1 SE). Groups with an asterisk are significantly different than the first clutch in the corresponding season.

order did not have independent effects on AE levels in egg yolk, but there was a significant season by clutch interaction (Table 2). During the first season, yolk AE was higher in eggs from the third clutch than it was in eggs from the first clutch (Fig. 3a). During the second

Table 3. Correlations among reproductive traits in female leopard geckos during their first and second breeding seasons ($n = 9$ for al correlations)

	Bivariate correlation	Partial correlation	Trait variance (s^2)
Season 1			
Female condition–Number of clutches	0.78**	0.96*** [0.87**]	Female condition = 0.31†
Female condition–Egg size	0.72*	0.95*** [0.79**]	Number of clutches = 3.75
Number of clutches–Egg size	0.17	−0.89*** [−0.65]	Egg size = 0.50
Season 2			
Female condition–Number of clutches	0.48	0.52 [0.57]	Female condition = 0.02
Female condition–Egg size	−0.13	0.27 [0.37]	Number of clutches = 2.78
Number of clutches–Egg size	−0.65	−0.68* [−0.65]	Egg size = 0.51
Between Seasons	Partial Correlation		
	Season 1 reproductive effort	Female condition (S2)	Number of clutches (S2)
Female condition (S2)	0.49	–	–
Number of clutches (S2)	−0.71*	0.67*	–
Egg size (S2)	−0.24	0.35	−0.63

*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001). Correlations in brackets are partial correlations using average egg size for the first two clutches rather than average egg size for the whole season. Variance in each trait is shown in the last column for each season: significant difference in variance between seasons is indicated (F-ratio$_{8,8} = 15.5$, †$P = 0.0004$). Partial correlations among total reproductive effort in the first season and reproductive traits in the second season are shown at the bottom of the table.

season, yolk AE was higher in the fourth and fifth clutches compared with the first clutch (Fig. 3a). There was also a significant female by clutch interaction for AE levels (Table 2). Breeding season and clutch order did not have independent effects on DHT levels in egg yolk, but there was a significant season by clutch interaction (Table 2). Yolk DHT was elevated in clutch 2 during the first breeding season but was similar among all the other clutches in both seasons (Fig. 3b). The concentration of E2 in egg yolk did not differ between seasons or change with clutch order (Table 2). Breeding season and clutch order did not have independent effects on T levels, but there was a significant season by clutch interaction (Table 2). Yolk T did not vary among clutches during the first season. However, yolk T was higher in clutch 3 than in clutch 1 in the second season (Fig. 3c).

Although we detected reproductive tradeoffs within and between breeding seasons, reproductive traits were not correlated with steroid levels in egg yolk (Table 4). The sole exception being a significant correlation between female condition and yolk AE in the second breeding season. Given the small sample size for correlations within individuals ($n = 9$), we also tested for relationships among residuals from REML ANOVA of female condition, egg size and yolk steroids. Residuals for each clutch of two eggs were averaged because these data points are not independent. Controlling for individual differences among females, breeding season, and the effect of clutch order, there was no correlation between female condition and AE levels in egg yolk ($r = -0.19$; $n = 42$; $P = 0.24$). In contrast, there was a negative correlation between female condition and DHT levels in egg yolk: females in poor condition deposited more DHT in their eggs than did females in good condition ($r = -0.42$; $n = 42$; $P = 0.005$; Fig. 4a). There was also a negative correlation between egg size and DHT levels in egg yolk: smaller eggs tended to have more DHT than larger eggs ($r = -0.38$; $n = 41$; $P = 0.01$; Fig. 4b).

Discussion

Here we present evidence for tradeoffs among several reproductive traits in female leopard geckos. Reproductive effort was strongly condition dependent in the first breeding season: females in good condition at the start of their first season produced larger eggs and more clutches than females in poor condition. Variation in female condition masked a significant tradeoff between egg size and egg number (i.e. number of clutches), exactly as predicted for capital breeders (Pease & Bull, 1988; Roff, 1992; Stearns, 1992; Bonnet *et al.*, 1998). Females lost mass and egg size decreased with consecutive clutches, further supporting the idea that stored resources were used to produce eggs during the first breeding season. Notwithstanding these results our data suggest that female leopard geckos were not strict capital breeders. First, clutch mass was greater than the mass females lost producing each clutch, which would occur if females also used income to produce eggs. In fact, females do not eat for 3–7 days before laying eggs, but are voracious after oviposition and feed during vitellogenesis (S. Simmons and D. Crews, personal observations). Secondly, females did not lose mass during the second season, yet they produced eggs that were on average 10% larger than eggs

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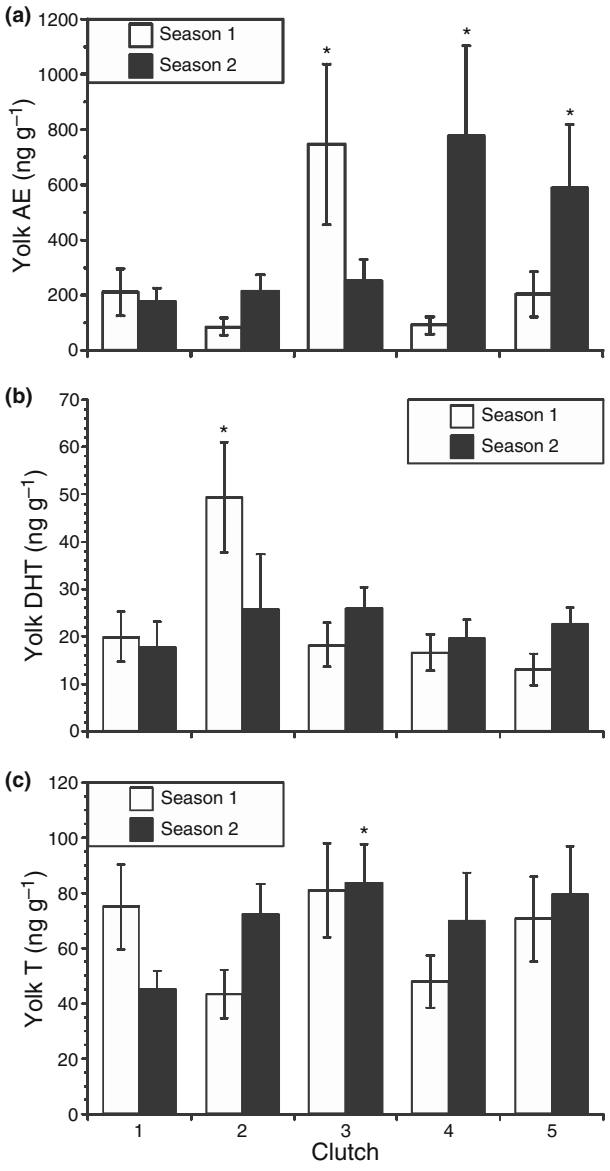

Fig. 3 Concentration of (a) androstenedione (AE), (b) dihydrotestosterone (DHT) and (c) testosterone (T) in egg yolk as a function of clutch number and breeding season. Steroid concentrations are least squares means (±1 SE). Groups with an asterisk are significantly different than the mean for the first clutch in the corresponding season.

in the first season. Finally, female condition did not influence reproductive effort in the second season as strongly as it did in the first season. Accordingly, the magnitude of the egg size–egg number tradeoff did not differ between bivariate and multivariate analyses in the second year. Together these results indicate that female leopard geckos used stored and acquired resources for egg production and that expenditure of capital vs. income varied both within and between breeding seasons.

Table 4. Bivariate correlations among reproductive traits and yolk steroids in female leopard geckos during their first and second breeding seasons ($N = 9$ for all correlations; $P < 0.05$).

	AE	DHT	E2	T
Season 1				
Female condition	0.18	−0.28	−0.12	0.37
Number of clutches	0.42	−0.20	−0.06	0.36
Average egg size	−0.16	−0.12	−0.24	0.06
Season 1				
Female condition	0.67*	−0.11	0.15	0.48
Number of clutches	0.08	0.47	−0.33	−0.34
Average egg size	0.02	−0.43	0.44	0.39

AE, androstenedione; DHT, dihydrotestosterone; E2, 17β-oestradiol; T, testosterone.

We also discovered a tradeoff between current and future reproduction. Females that invested heavily in reproduction in their first season produced fewer clutches in their second season even though they were in similar condition at the beginning of both seasons. This temporal tradeoff may be mediated by differences in growth as females that invested less in reproduction during their first season gained mass between seasons and produced more clutches in their second season. In discussing tradeoffs, we assume that female investment in each egg is proportional to egg mass. This is a realistic first assumption based on studies of egg components (i.e. water, protein and lipid content) and egg mass in other lizards. Vitellogenin and VLDLy comprise >95% of the lipid and protein content of egg yolk and account for *c.* 90% of the dry mass of lizard eggs (Speake & Thompson, 2000; Thompson & Speake, 2002). Having found a consistent tradeoff between egg size and egg number in both seasons, we were interested in assessing the idea that steroids mediate this tradeoff.

We hypothesized that steroids might influence the allocation of resources to egg size at the expense of egg numbers (i.e. number of clutches). For instance, E2 induces synthesis of vitellogenin and VLDLy in a dose-dependent manner in oviparous vertebrates (Means & O'Malley, 1972; Schneider, 1992; Burley *et al.*, 1993; Bujo *et al.*, 1997; Walzem *et al.*, 1999). Consequently, one might expect a positive correlation between egg mass (i.e. yolk mass) and E2 content of eggs. On the other hand, the number of clutches laid by females would be inversely related to the E2 content of their eggs. Despite the plausibility of these predictions, the number of clutches produced by female leopard geckos and average egg size were not related to levels of E2 deposited in egg yolk in either season. Moreover, there were no consistent changes in yolk E2 with clutch order or across breeding seasons. These findings lead us to conclude that E2 levels in egg yolk are not related to the egg size–egg number tradeoff. However, this conclusion does not imply that E2 does not regulate the tradeoff between egg size and

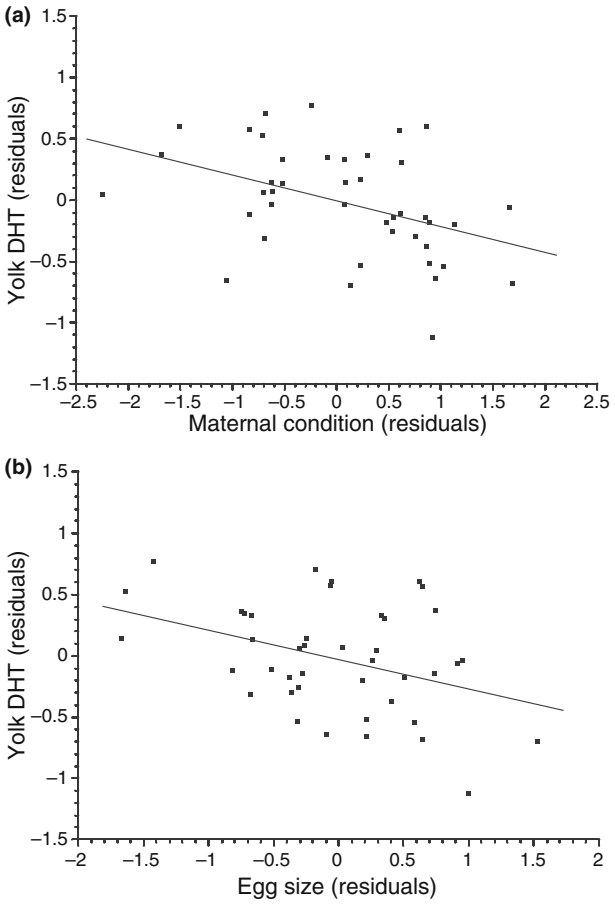

Fig. 4 (a) Plot of residuals for yolk dihydrotestosterone (DHT) concentration vs. residuals for female condition. (b) Plot of residuals for yolk DHT concentration versus residuals for egg size. Studentized residuals were from the statistical analyses described in the text: i.e. restricted maximum likelihood ANOVA with season, clutch and female ID as independent variables.

number because yolk steroids may not reflect steroid levels in the maternal circulation during egg development (see discussion below). Female condition was correlated with average levels of yolk AE in the second season but this may be a type I error; i.e. just one of the 24 correlations among reproductive traits and yolk steroids was significant (Table 4). Sample size was small for correlations within individuals so we also tested for correlations among residuals for female condition, egg size and yolk steroids.

Statistically controlling for individual, seasonal and clutch differences, females in relatively poor condition deposited more DHT in their eggs than females in better condition. Likewise, there was a negative correlation between residuals for egg size and residuals for yolk DHT. Smaller eggs tended to have higher DHT levels than larger eggs. These patterns are interesting from an evolutionary perspective because DHT was deposited in eggs at an apparent disadvantage due to decreased allocation of maternal resources (i.e. small egg size) and poor maternal condition. Androgens are preferentially allocated to disadvantaged eggs in other species. Female canaries, for instance, deposit increasing amounts of androgens in successive eggs within a clutch (Schwabl, 1993). Offspring from the last eggs in a clutch are significantly smaller than siblings from the first eggs because of hatching asynchrony. Nevertheless, elevated levels of androgens in the last eggs boost growth rate and increase social status of hatchling canaries (Schwabl, 1996a,b). Similar effects of yolk androgens on offspring growth and behaviour have been observed in red-winged blackbirds and black-headed gulls (Lipar & Ketterson, 2000; Eising *et al.*, 2001). Maternally derived androgens in egg yolk counteract the detrimental effects of hatching asynchrony in these birds and may play a comparable role in modifying offspring phenotype in leopard geckos. These findings preface our study of the relationship between maternal and yolk steroids.

Our study suggests maternal androgens do not simply diffuse into yolking follicles. Although T levels in egg yolk were proportional to T levels in the maternal circulation during late vitellogenesis, yolk T was not directly related to maternal T. In fact, yolk T was 1–2 orders of magnitude less concentrated than maternal T during late vitellogenesis and was unrelated to maternal T during early vitellogenesis. According to biophysical principles, one would expect T to diffuse down its concentration gradient and into the lipophilic yolk. Furthermore, there was an inverse relationship between yolk T and circulating levels of T after ovulation. Together these results indicate that T levels in egg yolk are regulated. Levels of DHT in egg yolk were also uncoupled from circulating levels of DHT during vitellogenesis. Although yolk DHT was proportional to maternal DHT after ovulation, there was again a large concentration gradient between yolk and circulating DHT (approximately four-fold higher DHT in the maternal circulation). Although lack of data on maternal AE levels prevent us from making direct comparisons with AE levels in egg yolk, yolk AE levels were correlated with yolk T levels. Moreover, maternal DHT and T displayed the same predictive relationship for yolk AE as for yolk T and yolk DHT (Table 1). Based on these results, it looks as if female leopard geckos regulate androgen levels in their eggs.

In contrast, E2 appears to diffuse between the maternal circulation and eggs in the oviduct. Most paired values for E2 fall near a theoretical line with a slope of one and an intercept near zero (dashed line in Fig. 1d), indicating a direct relationship between maternal E2 and yolk E2. In addition, this line is shifted slightly to the left, which would be expected if E2 were drawn into lipophilic yolk. We suspect that the two highest values for maternal E2 (see Fig. 1d) were collected from females immediately after ovulation and before E2 had dropped to levels

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typical of gravid females. Levels of E2 for these two females were similar to those found in females during late vitellogenesis when E2 is at its peak. These results are notable because only a handful of studies have examined the relationship between steroid levels in the maternal circulation and levels of maternally derived steroids in eggs. For example, levels of E2 and T in maternal plasma were not correlated with levels of E2 and T in yolking follicles of the green anole (Lovern & Wade, 2003). Yolk T therefore appears to be regulated in anoles and leopard geckos. However, these lizards differ with regard to yolk E2, which freely diffused between plasma and yolk in leopard geckos but was regulated in anoles. This species difference may be explained by a simple difference in sampling procedure: circulating and yolk E2 were measured in vitellogenic female anoles, whereas maternal and yolk E2 were only correlated for gravid leopard geckos.

Unfortunately, studies in birds do not provide a clearer picture of whether maternal steroids passively diffuse into eggs or if steroid levels in eggs are actively regulated (Groothuis & von Engelhardt, 2005; Groothuis *et al.*, 2005). Whereas positive correlations between maternal and yolk steroids have been observed in some species (Schwabl, 1996b; Groothuis and von Engelhardt, 2005), negative or no correlations have been reported in others (Mazuc *et al.*, 2003; Verboven *et al.*, 2003; Tanvez *et al.*, 2004). There is even conflicting data within the same species (Schwabl, 1996b; Marshall *et al.*, 2005). In addition, there has been little mention of steroid transfer between the maternal circulation and eggs in the oviduct as we observed for E2 in leopard geckos (but see Painter *et al.*, 2002). Despite this uncertainty, most papers on yolk steroids refer to 'maternal allocation' of steroids as if it were an active, regulated process. Carefully designed sampling protocols are necessary to control for the dynamic nature of female reproductive physiology and the potential for steroid transfer throughout the female reproductive tract. Another caveat is that we focused on the first two years of reproduction and did not follow females through their entire reproductive lifespan. Given that leopard geckos can reproduce for at least 6–8 years and that there are often age-related changes in hormonal regulation of reproduction in vertebrates, age-related changes in the transfer of maternal steroids to eggs may be an important source of variation.

In summary, we detected tradeoffs among key reproductive traits using multivariate statistics. These results are in agreement with predictions from life history theory, but also indicate that the nature of tradeoffs can vary within species. In particular, use of stored vs. acquired resources for egg production varied both within and between breeding seasons in female leopard geckos. A tradeoff between egg size and number was found in both seasons but was not associated with steroid levels in egg yolk, presumably because yolk steroids did not reflect steroid levels in the maternal circulation when eggs were yolking. Nevertheless, females in poor condition deposited more DHT in their eggs than females in good condition. Likewise, more DHT was allocated to smaller eggs than to larger eggs. These findings suggest that maternally derived androgens may adaptively modify offspring phenotype in leopard geckos as they do in several bird species.

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