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Abstract

Corticosterone exposure during prenatal development as a result of maternal upregulation of circulating hormone levels has been shown to have effects on offspring development in mammals. Corticosterone has also been documented in egg yolk in oviparous vertebrates, but the extent to which this influences phenotypic development is less studied. We show that maternal corticosterone is transferred to egg yolk in an oviparous lizard (the mallee dragon, Ctenophorus fordi Storr), with significant variation among clutches in hormone levels. Experimental elevation of yolk corticosterone did not affect hatching success, incubation period or offspring sex ratio. However, corticosterone did have a sex-specific effect on skeletal growth during embryonic development. Male embryos exposed to relatively high levels of corticosterone were smaller on average than control males at hatching whereas females from hormone-treated eggs were larger on average than control females. The data thus suggest that males are not just more sensitive to the detrimental effects of corticosterone but rather that the sexes may have opposite responses to corticosterone during development. Positive selection on body size at hatching for both sexes in this species further suggests that increased corticosterone in egg yolk may have sex-specific fitness consequences, with potential implications for sex allocation and the evolution of hormone-mediated maternal effects.

Keywords

corticosterone, yolk, lizard, response, developmental, oviparous, sex, specific, plasticity

Disciplines

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Sex-specific developmental plasticity in response to yolk corticosterone in an oviparous lizard

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SUMMARY

Corticosterone exposure during prenatal development as a result of maternal upregulation of circulating hormone levels has been shown to have effects on offspring development in mammals. Corticosterone has also been documented in egg yolk in oviparous vertebrates, but the extent to which this influences phenotypic development is less studied. We show that maternal corticosterone is transferred to egg yolk in an oviparous lizard (the mallee dragon, *Ctenophorus fordi* Storr), with significant variation among clutches in hormone levels. Experimental elevation of yolk corticosterone did not affect hatching success, incubation period or offspring sex ratio. However, corticosterone did have a sex-specific effect on skeletal growth during embryonic development. Male embryos exposed to relatively high levels of corticosterone were smaller on average than control males at hatching whereas females from hormone-treated eggs were larger on average than control females. The data thus suggest that males are not just more sensitive to the detrimental effects of corticosterone but rather that the sexes may have opposite responses to corticosterone during development. Positive selection on body size at hatching for both sexes in this species further suggests that increased corticosterone in egg yolk may have sex-specific fitness consequences, with potential implications for sex allocation and the evolution of hormone-mediated maternal effects.

Key words: *Ctenophorus fordi*, hormones, phenotypic plasticity.

INTRODUCTION

Hormones play an important role in developmental plasticity (Dufty et al., 2002; West-Eberhard, 2003). For example, steroids produced during embryonic development induce and regulate differences between the sexes in primary sexual characters, brains and behaviour (Adkins-Regan, 2005; Carere and Balthazart, 2007). This suggests that hormone production by embryos will be highly time and site specific and subject to strong selection to avoid production of inferior (for example, intermediate) phenotypes. However, embryos are also subject to hormone exposure from external sources, which may or may not be adaptive. In rodents, for example, a by-product of embryonic steroid production is that hormone leakage between foetuses can modify the absolute or relative hormone exposure of individual embryos (Ryan and Vandenbergh, 2002). Such variation in hormone exposure has been implied in causing morphological, physiological, behavioural and life-history variation among offspring in both mammals and viviparous lizards (reviewed by Clark and Galef, 1998; Ryan and Vandenbergh, 2002; Uller, 2006).

Increases in maternal hormone profiles during reproduction also have the potential to affect hormone exposure of offspring. For example, maternal up-regulation of glucocorticoids (Wingfield and Kitayski, 2002) frequently leads to changes in offspring development, including decreased birth weight, increased anxiety and impaired coping abilities [i.e. the prenatal stress syndrome (Welberg and Seckl, 2001)]. Although this is commonly assumed to be detrimental to offspring fitness, stress-mediated maternal modulation of phenotypic development could also represent an evolved strategy that maximizes survival of offspring under certain environmental conditions [adaptive *vs* non-adaptive maternal effects (Marshall and Uller, 2007; Müller et al., 2007; Uller, 2008)].

The prenatal stress syndrome is often assumed to result from a direct action of glucocorticoids on embryonic development (Welberg and Seckl, 2001) but it is very difficult to disentangle direct effects of maternal glucocorticoids on offspring development from other changes in the maternal–foetal relationship that occur during maternal stress (Uller and Olsson, 2006a). However, studies of oviparous vertebrates have shown that maternal hormones are transferred to egg yolk, which provides a system in which the direct effect of hormones can be experimentally assessed (Groothuis et al., 2005). Perhaps surprisingly, given the extensive literature on the prenatal stress syndrome, the vast majority of work on oviparous species has been conducted on yolk androgens [which have been shown to have both short- and long-term consequences for offspring (reviewed by Groothuis et al., 2005)], whereas corticosterone has largely been ignored [exceptions (McCormick, 1998; McCormick, 1999; Love et al., 2005; Hayward et al., 2006; Love et al., 2008)]. However, if corticosterone is indeed transferred to the egg yolk, this represents an important complementary system in which to evaluate the adaptive nature of maternal programming of offspring during maternal stress for several reasons. First, studies of maternal transfer of corticosterone to egg yolk could provide insight into the general developmental responses to corticosterone exposure *per se*, rather than maternal effects that are secondary products of increased maternal corticosteroids. Second, in contrast to mammals, transfer of corticosterone is constrained to occur during egg formation only, which may reduce the opportunity for precise and context-dependent allocation in response to perceived selection on offspring (Uller,

2008). Third, the absence of sudden and unpredictable hormone exposure at different stages in development of embryos in oviparous species could have influenced the ability to ignore, or capitalize on, hormonal cues.

The roles of maternal hormone transfer as adaptation or constraint are particularly important when hormones have sex-specific fitness consequences, as this can lead to sex-specific maternal effects, select for differential sex allocation and, ultimately, exercise selection on sex determination (reviewed by Groothuis et al., 2005; Uller, 2006; Carere and Blathazart, 2007; Rutkowska and Badyaev, 2008; Uller and Badyaev, 2009). For example, males are commonly more strongly affected by prenatal stress (Welberg and Seckl, 2001), and increased maternal corticosterone levels have been experimentally shown to bias sex ratios of offspring in some birds (e.g. Pike and Petrie, 2006). This could be adaptive if it reduces the fitness reduction associated with combination of male phenotypes and high corticosterone exposure *in ovo* (Love et al., 2005).

In the present paper, we provide descriptive data on yolk corticosterone in an oviparous lizard, the mallee dragon *Ctenophorus fordi,* and use experimental manipulation of yolk corticosterone to assess its consequences for offspring development and phenotype at hatching. Based on previous data on mammals, birds and fish, we expected to find maternal transfer of corticosterone and that it would have negative consequences on offspring development and survival, in particular for males.

MATERIALS AND METHODS

The mallee dragon, *Ctenophorus fordi* Storr, is a small [~40–60 mm snout to vent length (SVL)] agamid lizard common to arid and semi-arid habitats in southern Australia. It emerges from hibernation in late August to mid September, and females produce between one and four clutches of two to five eggs [the large majority of clutches having two eggs only (Uller and Olsson 2006b; Uller and Olsson, 2009)] over the reproductive season, which represents the total lifetime reproductive output for the majority of individuals as survival into the second year is low (Cogger, 1978). The mating system is polygynandrous and males are not territorial.

Gravid female mallee dragons from a population in Yathong Nature Reserve, NSW, were captured by noosing just before oviposition over the reproductive season in 2005 for assessment of natural variation in yolk hormones. They were housed individually in cages (645×413×347 mm) until oviposition according to standard procedures at the University of Wollongong (for details, see Uller and Olsson, 2006b; Uller et al., 2007). Eggs were sampled within 12 h of oviposition, weighed to the nearest 0.01 g and immediately frozen for future hormone assays (see below). All eggs from nine clutches and one single egg from an additional 16 clutches were sampled for analyses of yolk testosterone and corticosterone content (N=38), which was subsequently used as reference for experimental elevation of yolk hormones in 2007 (see below).

In 2007, gravid females from the same population were captured and housed as above. However, rather than being frozen, each experimental egg was injected with 200 pg of corticosterone dissolved in 1 µl sesame oil, and each control egg was injected with 1 µl sesame oil. We alternated clutches so that every second clutch received hormone injections and the others received vehicle injection only. The injected amount of corticosterone represents approximately 1.5–3 standard errors of the mean amount of corticosterone in *C. fordi* eggs and consequently should correspond well to naturally high levels (and is well below the maximum hormone levels found in eggs in the present study; see Results). The injection was performed through the most pointed end of the egg, and the needle was inserted approximately one-third into the egg yolk. Eggs were incubated individually in plastic cups with sterilized vermiculite mixed with sterilized water (volume ratio 7:1) and incubated at 29°C, which has been shown to minimize embryonic mortality in this species (Uller et al., 2008). Incubators were checked daily for hatchlings, which were measured (SVL and total length to the nearest 0.5 mm), weighed (to the nearest 0.01 g) and sexed using eversion of the hemipenes (Harlow, 1996).

Hormone assay

Lizard eggs were weighed and the shell removed, after which they were weighed separately to determine mass of the egg yolk [albumen forms a very minor part of mallee dragon eggs (for a review, see Thompson and Speake, 2004)]. The contents of each egg were placed in a glass tube containing 500 µl of EIA assay buffer (Cayman Chemical, Ann Arbor, MI, USA) and homogenised using glass beads. Each tube was spiked with tritiated corticosterone (approximately 2000 c.p.m. (75 Bq) in 20 µl; Amersham, Buckinghamshire, UK) to allow calculation of post-extraction hormone recovery. Contents of tubes were mixed and incubated at 4°C overnight. Testosterone (T) and corticosterone (CORT) were extracted from egg contents based on the methods of Schwabl (Schwabl, 1993). Briefly, each sample was extracted twice for 90 min in 3 ml of a 30:70 (v:v) mixture of petroleum ether and diethyl ether. Ether extracts were decanted after freezing the aqueous fraction in methanol-dry ice. The two extracts were combined, dried over a stream of nitrogen gas, reconstituted in 50 ml ethanol, diluted 1:5 in buffer and stored at –20°C until assayed.

Testosterone and corticosterone levels were measured using Cayman EIA assay kits (#582701 and #500651, respectively). All samples were assayed on a single plate, thus eliminating interplate variation. In addition, a 50 µl aliquot of each sample extract was counted in a beta counter to determine percent recovery of the tritiated corticosterone spike. Because our preliminary tests showed very low concentrations of testosterone and corticosterone in lizard eggs, we opted not to purify steroids by column chromatography, potentially reducing concentrations further. Thus, we only determined recovery efficiency in individual samples for corticosterone, and final corticosterone levels were adjusted to account for individual sample recovery. To account for extraction loss of testosterone we applied an average 65% recovery to all eggs based on our previous analyses of testosterone in agamid eggs (L.A., unpublished data).

RESULTS

Out of the 38 eggs that were analyzed, 20 contained detectable amounts of corticosterone and 24 of testosterone (means ± s.e.m. per egg: T, 28.9±5.76 pg; CORT, 117.3±66.8 pg) (excluding eggs with no detectable hormones: T, 45.8±7.15 pg; CORT, 354.7±121.90 pg). Analyzing only the nine clutches for which we had more than one egg revealed significant differences among clutches in both yolk corticosterone and testosterone (Kruskal–Wallis test; χ^2=19.0, P=0.021 and χ^2=17.7, P=0.024, respectively). There was a significant positive correlation between the total amount of corticosterone and testosterone (using means per clutch for clutches for which we had more than one egg; r=0.83, P<0.001) whereas neither of the hormones was correlated with egg size (both P>0.40) or oviposition date (both P>0.2). Omitting clutches for which we could not find any detectable amounts of hormones resulted in similar patterns (r=0.73, P=0.008), as did the removal of an outlier (T=105.6, CORT=1836.0; r=0.64, P=0.035) (Fig. 1). However, it should be noted that this outlier

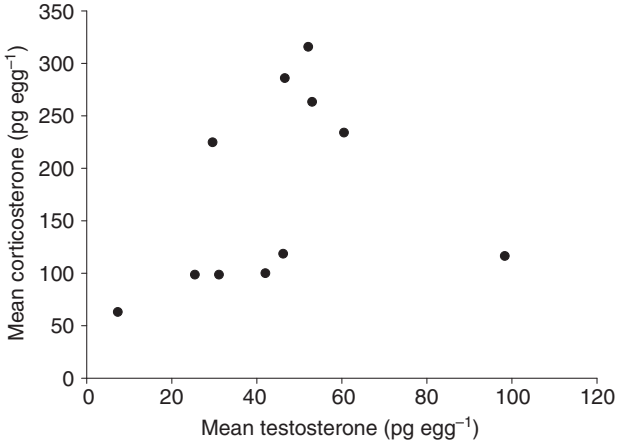

Fig. 1. Correlation between mean yolk testosterone and corticosterone content in eggs of the mallee dragon, *Ctenophorus fordi*. Only clutches where hormones were detected in at least one egg are included, and one outlier has been removed (see text for details).

represented the mean of two eggs, both of which had very high hormone content, and therefore is likely to reflect biologically relevant levels.

An experimental increase in yolk corticosterone did not have a significant effect on egg mortality or offspring sex ratio (logistic regressions; χ^2=1.21, P=0.26 and χ^2=0.48, P=0.49, respectively; hatching success was 85.4% and 90.8% for corticosterone-treated and control eggs, respectively). A linear mixed model with sex and treatment as fixed factors, clutch identity as a random factor and egg mass as a covariate showed no significant effect of sex, treatment or egg mass on incubation time (all P>0.5). Furthermore, there was no overall significant effect of the treatment on hatchling mass, SVL or total length, nor were there any interactive effects with egg mass (Table 1). However, there was a significant interaction between offspring sex and egg treatment on measures of offspring size at hatching, which resulted from an opposite effect of yolk corticosterone on males and females. Males from corticosterone-injected eggs were smaller and lighter than males from control eggs, whereas females from corticosterone eggs were larger and heavier than control females (Table 1; Fig. 2). *Post-hoc* tests failed to reveal a significant effect of treatment within each sex (all P>0.05), but statistical power is reduced as a result of relatively small sample sizes. The interaction effect on body mass disappeared when we controlled for skeletal size (i.e. SVL; treatment × sex; $F_{1,76.5}$=2.22, P=0.14).

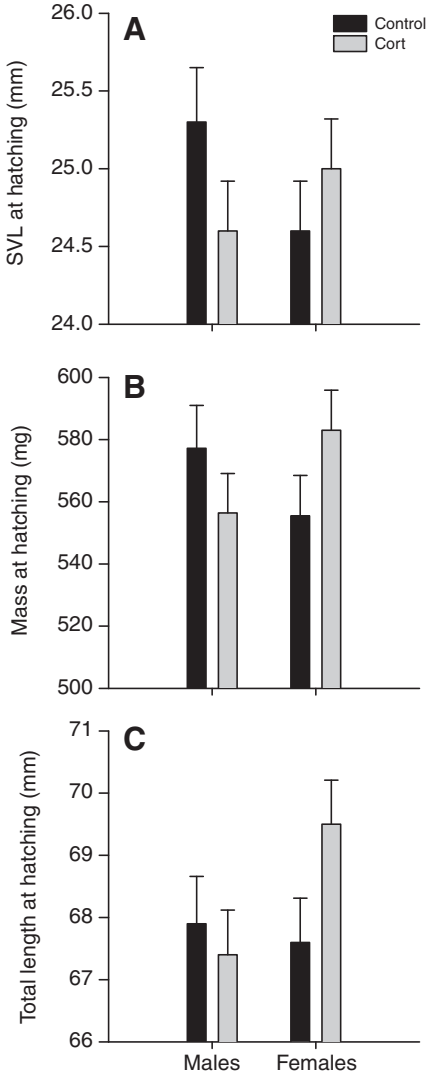

Fig. 2. (A) Snout–vent length (SVL), (B) offspring body mass and (C) total body length at hatching. Data represent least square means (± s.e.m.) from linear mixed models, as in Table 1.

DISCUSSION

Our data show that corticosterone of maternal origin is present in egg yolk of the mallee dragon, *Ctenophorus fordi*, and that it has sex-specific consequences for offspring development with respect to skeletal development, i.e. body length. Whereas males from

Table 1. Linear Mixed Models (PROC MIXED, SAS v. 9.1) of body mass at hatching, snout–vent length (SVL) at hatching and total length at hatching

	Body mass		SVL		Total length	
Random effects	Estimate		Estimate		Estimate	
Clutch ID (treatment)	2296		1.22		5.58	
Residual	752		0.63		3.32	
Fixed effects	d.d.f.	*F*	d.d.f.	*F*	d.d.f.	*F*
Treatment	36.6	0.07	34	0.23	35.7	2.45
Sex	57.2	0.04	63.2	0.67	66.2	0.72
Treatment × sex	57.0	7.02*	63.1	4.90*	66.1	4.66*
Egg mass	61.9	39.9***	53.2	7.07*	52.7	13.41***

*P<0.05, **P<0.01, ***P<0.001.

corticosterone-injected eggs showed an average decrease in SVL and total length, females showed an average increase. The data therefore suggest that males are not just more sensitive to corticosterone exposure but rather that the sexes have opposite responses to corticosterone exposure during development. We discuss these results in relation to the evolution of hormone-mediated maternal effects and sex allocation.

There was a large variation among clutches in corticosterone content. Furthermore, yolk corticosterone and testosterone showed a positive covariation. Whereas testosterone can be locally produced in the ovary, corticosteroid concentration in eggs has been suggested to reflect passive transfer from plasma to eggs, with little scope for regulation (Groothuis and Schwabl, 2008). Regardless, it is clear that maternal corticosterone can be found in bird, lizard and fish egg yolk in sufficiently high levels to affect offspring development (e.g. McCormick, 1999; Hayward and Wingfield, 2004), although its consequences for offspring development in oviparous animals are poorly understood.

Administration of corticosterone to mothers, or increased maternal stress, in viviparous species induces a number of different effects in the offspring (reviewed by Weinstock, 2001; Welberg and Seckl, 2001). Nevertheless, in viviparous animals it is unclear whether such effects are directly caused by embryonic exposure to steroids transferred from the mother or by other changes in maternal physiology resulting from increased corticosterone levels [for discussion, see Uller and Olsson (Uller and Olsson, 2006a)]. For example, studies of both mammals and viviparous lizards have found effects on offspring size, body condition and behaviour of juveniles (e.g. de Fraipont et al., 2000; Welberg and Seckl, 2001; Meylan and Clobert, 2005). However, experimental manipulation of corticosterone exposure of offspring *via* hormone injection into eggs in the ovo-viviparous lizard *Lacerta vivipara* suggested that only behavioural changes are directly caused by corticosterone [although sex-specific effects were not considered (Uller and Olsson, 2006a)]. Oviparous vertebrates allow an alternative system in which to explore these effects and their adaptive significance, which may differ compared with viviparous species where the maternal–foetal relationship is both more intimate and prolonged. Furthermore, there is substantial variation in the length of intrauterine retention of eggs in squamate reptiles [mallee dragons being at the very early stage (Uller et al., 2007)], which may be of interest from a comparative perspective on maternal stress during reproduction and its effects on offspring development.

In oviparous vertebrates, glucocorticosteroid exposure during development has been shown to affect behaviour (Hayward and Wingfield, 2006), growth and mortality (McCormick, 1999; Hayward and Wingfield, 2004; Warner et al., 2009) (see also Sinervo and DeNardo, 1996; Love et al., 2005) and there is some evidence for sex-specific plasticity in quail (Hayward et al., 2006). Furthermore, high levels of prenatal corticosterone seem to reduce survival of male embryos in the jacky dragon [*Amphibolurus muricatus* (Warner et al., 2009)] but increase survival of male (but not female) offspring post-parturition in the viviparous common lizard [*Lacerta vivipara* (Meylan and Clobert, 2005)]. Despite the fact that corticosterone exposure generally reduces size at hatching or birth and that males seem to be more strongly affected, the positive effect on body size in females in our study, and the contrasting results for other lizards [including another Australian agamid (Warner et al., 2008)], suggests that developmental plasticity in response to corticosterone can evolve. Interestingly, a positive effect on size in female, but not male, hatchlings was also found in corticosterone-implanted female *Uta stansburiana* (Sinervo and DeNardo, 1996), which could represent convergent evolution *via* selection on sex-specific sensitivity.

Size at hatching is under positive selection in the mallee dragon *via* a positive effect on survival and size at the onset of breeding (T.U. and M.O., unpublished data). Thus, transfer of corticosterone [and testosterone (Uller et al., 2007)] to the egg yolk and prenatal sensitivity to hormone exposure should be under selection. Whereas the evidence for sex-specific effects of yolk testosterone in this species is indirect [*via* sex-specific fitness consequences of size at maturity (Uller and Olsson, 2006b; Uller et al., 2007)], the present study clearly shows that the sexes may respond differently to increased corticosteroids in terms of size at hatching and potentially survival *via* the link between hatchling size and survival to maturation. The small within-clutch variation in yolk steroids and lack of sex ratio adjustment (Uller and Olsson, 2006b; Uller et al., 2008) suggest that sex-specific hormone allocation does not occur in *C. fordi* [possibly because of similar oocyte exposure to hormones as a result of overlapping growth (Badyaev et al., 2006; Uller, 2006)], despite the fact that the present study may indicate sex-specific optima. However, it should be pointed out that results from phenotypic engineering studies must be interpreted with caution as they only identify direct effects of the manipulated factor (in this case, yolk corticosterone), not the potential ways in which such effects could be accommodated developmentally and evolutionarily. For example, it has been suggested that yolk antioxidants can compensate for detrimental effects of high testosterone exposure *in ovo* (e.g. Royle et al., 2001), and similar pre- or post-natal compensatory mechanisms may exist for corticosterone, for example *via* (sex-specific) behavioural adjustment or growth (Meylan and Clobert, 2005; Uller and Olsson, 2006a). Furthermore, maternal effects may be environment dependent, in particular if they have evolved to enable a match between offspring phenotype and offspring environment (Love et al., 2005; Uller, 2008). Ultimately, combining correlative and experimental studies with estimates of fitness under ecologically relevant contexts is required to understand the causes and consequences of hormone-mediated maternal effects (Groothuis et al., 2005; Müller et al., 2007; Groothuis and Schwabl, 2008; Uller, 2008).

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REFERENCES

Adkins-Regan, E. (2005). *Hormones and Animal Social Behavior.* Princeton, NJ: Princeton University Press.

Badyaev, A. V., Seaman, D. A., Navara, J. K., Hill, G. E. and Mendonca, M. T. (2006). Evolution of sex-biased maternal effects in birds: III. Adjustment of ovulation order can enable sex-specific allocation of hormones, carotenoids, and vitamins. *J. Evol. Biol.* **19**, 1044-1057.

Carere, C. and Balthazart, J. (2007). Sexual versus individual differentiation: the controversial role of avian maternal hormones. *Trends Endocrinol. Metab.* **18**, 73-80.

Clark, M. M. and Galef, B. G., Jr (1998). Perinatal influences on the reproductive behavior of adult rodents. In *Maternal Effects as Adaptations* (ed. T. A. Mousseau and C. W. Fox), pp. 261-271. New York: Oxford University Press.

Cogger, H. G. (1978). Reproductive cycles, fat body cycles and socio-sexual behaviour in the mallee dragon, *Amphibolurus fordi* (Lacertilia: Agamidae). *Aust. J. Zool.* **26**, 653-672.

de Fraipont, M., Clobert, J., John-Alder, H. and Meylan, S. (2000). Increased pre-natal corticosterone promotes philopatry of offspring in common lizards *Lacerta vivipara*. *J. Anim. Ecol.* **69**, 404-419.

Dufty, A. M., Clobert, J. and Møller, A. P. (2002). Hormones, developmental plasticity and adaptation. *Trends Ecol. Evol.* **17**, 190-196.

Groothuis, T. G. G. and Schwabl, H. (2008). Hormone-mediated maternal effects in birds: mechanisms matter but what do we know of them? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **363**, 1647-1661.

Groothuis, T. G. G., Müller, W., von Engelhardt, N., Carere, C. and Eising, C. (2005). Maternal hormones as a tool to adjust offspring phenotype in avian species. *Neurosci. Biobehav. Rev.* **29**, 329-352.
Harlow, P. S. (1996). A harmless technique for sexing hatchling lizards. *Herpetol. Rev.* **2**, 71-72.
Hayward, L. S. and Wingfield, J. C. (2004). Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *Gen. Comp. Endocrinol.* **135**, 365-371.
Hayward, L. S., Richardson, J. B., Grogan, M. N. and Wingfield, J. C. (2006). Sex differences in the organizational effects of corticosterone in the egg yolk of quail. *Gen. Comp. Endocrinol.* **146**, 144-148.
Love, O. P., Chin, E. H., Wynne-Edwards, K. E. and Williams, T. D. (2005). Stress hormones: a link between maternal condition and sex-biased reproductive investment. *Am. Nat.* **166**, 751-766.
Love, O. P., Wynne-Edwards, K. E., Bond, I. and Williams, T. D. (2008). Determinants of within- and among-clutch variation in yolk corticosterone in the European starling. *Horm. Behav.* **53**, 104-111.
Marshall, D. J. and Uller, T. (2007). When is a maternal effect adaptive? *Oikos* **166**, 1957-1963.
McCormick, M. I. (1998). Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. *Ecology* **79**, 1873-1883.
McCormick, M. I. (1999). Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. *Oecologia* **118**, 412-422.
Meylan, S. and Clobert, J. (2005). Is corticosterone-mediated phenotype development adaptive? Maternal corticosterone treatment enhances survival in male lizards. *Horm. Behav.* **48**, 44-52.
Mousseau, T. A. and Fox, C. W. (1998). *Maternal Effects as Adaptations.* New York: Oxford University Press.
Müller, W., Lessells, M., Korsten, P. and von Engelhardt, N. (2007). Manipulative signals in family conflict? On the function of maternal yolk hormones in birds. *Am. Nat.* **169**, E84-E96.
Pike, T. W. and Petrie, M. (2006). Experimental evidence that corticosterone affects offspring sex ratios in quail. *Proc. Biol. Sci.* **273**, 1093-1098.
Royle, N. J., Surai, P. F. and Hartley, I. R. (2001). Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects? *Behav. Ecol.* **12**, 381-385.
Rutkowska, J. and Badyaev, A. V. (2008). Meiotic drive and sex determination: molecular and cytological mechanisms of sex ratio adjustment in birds. *Philos. Trans. R. Soc. B Biol. Sci.* **363**, 1675-1686.
Ryan, B. C. and Vandenbergh, J. G. (2002). Intrauterine position effects. *Neurosci. Biobehav. Rev.* **26**, 665-678.
Schwabl, H. (1993). Yolk is a source of maternal testosterone for developing birds. *Proc. Natl. Acad. Sci. USA* **90**, 11446-11450.
Sinervo, B. and DeNardo, D. (1996). Costs of reproduction in the wild: path analysis of natural selection and experimental tests of causation. *Evolution* **50**, 1299-1313.
Thompson, M. B. and Speake, B. K. (2004). Egg morphology and composition. In *Reptilian Incubation, Environment, Evolution and Behaviour* (ed. D. C. Deeming), pp. 45-74. Nottingham: Nottingham University Press.
Uller, T. (2006). Sex-specific sibling interactions and offspring fitness in vertebrates: patterns and implications for maternal sex ratios. *Biol. Rev.* **18**, 207-217.
Uller, T. (2008). Developmental plasticity and the evolution of parental effects. *Trends Ecol. Evol.* **23**, 432-438.
Uller, T. and Badyaev, A. V. (2009). Evolution of "determinants" in sex-determination: a novel hypothesis for the origin of environmental contingencies in avian sex bias. *Semin. Cell Dev. Biol.* (in press).
Uller, T. and Olsson, M. (2006a). Direct exposure to corticosterone during embryonic development influences behaviour in an ovoviviparous lizard. *Ethology* **112**, 390-397.
Uller, T. and Olsson, M. (2006b). No seasonal sex ratio shift despite sex-specific fitness returns of hatching date in a lizard with genotypic sex determination. *Evolution* **60**, 2131-2136.
Uller, T. and Olsson, M. (2009). Offspring size-number trade-off in a lizard with small clutch sizes: tests of invariants and potential implications. *Evol. Ecol.* (in press).
Uller, T., Astheimer, L. and Olsson, M. (2007). Consequences of maternal yolk testosterone for offspring development and survival: experimental test in a lizard. *Funct. Ecol.* **21**, 544-551.
Uller, T., Odierna, G. and Olsson, M. (2008). Sex ratio variation and sex determination in the mallee dragon, *Ctenophorus fordi* (Lacertilia:Agamidae) *Integr. Zool.* **3**, 157-165.
Warner, D. A., Radder, R. S. and Shine, R. (2009). Corticosterone exposure during embryonic development affects offspring growth and sex ratios in opposing directions in two lizard species with environmental sex determination. *Physiol. Biochem. Zool.* (in press).
Weinstock, M. (2001). Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Prog. Neurobiol.* **65**, 427-451.
Welberg, L. A. M. and Seckl, J. R. (2001). Prenatal stress, glucocorticoids and the programming of the brain. *J. Neurobiol.* **13**, 113-128.
West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution.* New York: Oxford University Press.
Wingfield, J. C. and Kitaysky, A. S. (2002). Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? *Integr. Comp. Biol.* **42**, 600-609.