Prenatal Sex Ratios Influence Sexual Dimorphism in a Reptile

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ABSTRACT   The prenatal environment influences offspring traits in a variety of ways and in a wide range of taxa. For example, maternal allocation of steroids to the eggs influences offspring traits in birds, and in some mammals the intrauterine position influences morphological, behavioural, and physiological traits due to sex-related steroid transfer between sibling fetuses. We show that similar phenomena occur in the common lizard (*Lacerta vivipara*), a viviparous reptile. Females developing in male-biased clutches had a more masculine allometry (relatively larger heads) at parturition than females developing in female-biased clutches. Males were correspondingly feminized in female-biased clutches. The effects could either be due to diffusion of steroids produced by the offspring or by a general tendency for females to allocate steroids according to the sex ratio of her clutch. Subsequent to parturition, the sexes differed in their growth trajectories depending on sex ratio environment. In males, the difference in allometry between sex ratio environments remained over time, whereas in females the corresponding effect disappeared. *J. Exp. Zool. 295A:183–187, 2003.* © 2003 Wiley-Liss, Inc.

Maternal effects play a significant role during development in shaping a variety of offspring characteristics (Bernardo, '96; Mousseau and Fox, '98). For example, egg size is closely related to neonate size in many species of fish, amphibians, reptiles, and birds (reviewed in Mousseau and Fox, '98), which also impact on offspring physiological performance at hatching (Sinervo, '90). However, not only nutrients but also hormones may be maternally transferred to the embryo. In birds, this has been demonstrated to result in steroid-induced post-hatching growth and behaviour effects (Schwabl, '93, '96).

In addition to maternally related hormonal effects, interactions between offspring during gestation could also influence offspring phenotype. In some mammals, the intrauterine position relative to siblings of the opposite sex has been demonstrated to affect morphology, behaviour, and life history characteristics of an offspring (Clark and Galef, '98). For example, in laboratory mice, females situated between two male fetuses had significantly longer anogenital distance (as do males) and were more aggressive than females developing between two female fetuses (Gandelmann et al., '77; vom Saal, '89). Some of these effects can even remain into adulthood and influence the sex ratio of a masculinized female's litter (Clark et al., '93; Vandenbergh and Huggett, '94). The reason the intrauterine position influences offspring traits is that androgens secreted by males late in gestation diffuse through the amniotic fluid and across the fetal membranes to adjacent fetuses (Even et al., '92). So far, this phenomenon has mainly been described for a small number of mammals (mainly rodents) in captivity (Clark and Galef, '98). Even though it seems reasonable to believe that similar phenomena occur in other viviparous taxa, few studies have investigated the effect of gestation environment on offspring phenotype, in particular in nonmammalian taxa (Clark and Galef, '95; Osypka and Arnold, 2000).

Although sibling interactions during pregnancy can have profound effects on offspring phenotype independently of genetic and maternal effects, its evolutionary consequences have attracted relatively little attention. For example, production of a biased clutch could sexually "neutralize" offspring, which may make them less attractive to partners and less competitive for resources (Clark et al., '92), as well as influence sex-specific life history characteristics, such as dispersal and sex allocation (Holekamp et al., '84; Clark et al., '93; Vandenberg and Huggett, '95).

In this study, we investigated prenatal sibling interactions in a viviparous, nonmammalian model, the common lizard (*Lacerta vivipara*), which is a reptile with a prolonged gestation period and genetic sex determination. Although the placenta is not as developed as in some other reptilian species with pronounced matrotrophy (Yaron, '85), the placental connection between the female and offspring allows diffusion of electrolytes (Panigel, '56) and most likely steroids, since transfer of these molecules has been demonstrated in other amniotes (Gandelmann et al., '77; vom Saal, '89).

MATERIALS AND METHODS

The common lizard (*Lacerta vivipara*) is a small (3–4 g), 50–70 mm snout-vent length (SVL), ground-dwelling lizard. It has one of the largest distributions of all reptilian species, occurring in most parts of Eurasia. In most of its range, it is live-bearing and has a mean clutch size of five young (range 2–12).

Fifty-six female *L. vivipara* were caught at four localities in south-western and at two localities in northern Sweden during May–June 2001. The females were mated in the field, as evident from copulation scars on the belly (inflicted by the male during copulation, Bauwens and Verheyen, '85). All animals were brought to the University of Göteborg subsequent to capture, where they were kept in 500 × 400 × 350 mm cages (4–5 lizards per cage), with the cages randomly distributed on shelves in a three level rack. The cages had peat and bark as bottom substrate, rocks and tiles as shelter, and a 40 W spotlight to allow thermoregulation. Crickets (*Gryllus* sp.) and mealworms (*Tenebrio* sp.) were provided to satiety and water was given ad libitum. Prior to parturition, females were kept separately for accurate scoring of maternity. Cages were checked at least twice daily for hatchlings. At parturition, the neonates were weighed to the nearest mg, measured snout to vent (SVL) and total length to the nearest 0.5 mm, and head width and head length were measured to the nearest 0.01 mm. Sex was determined by hemipenis eversion, which has been confirmed to accurately establish sexual identity in repeatability trials in a variety of reptilian taxa (Harlow, '96; Olsson and Shine, '99). The neonates were kept in cages as described for adults with small crickets (*Gryllus* sp.) and *Drosophila* provided ad libitum. Four days post-parturition, the neonates were weighed again and transferred to small, individual cages in thermal incubators at two different light regimes, 18:6 L:D and 6:18 L:D. In the light phase, the lizards were maintained at 30°C, which is the preferred body temperature for common lizards (Van Damme et al., '87). In the dark phase, temperature was kept at 15°C. The experiment followed a split-brood design (Via, '93), with half of the young per clutch allocated to each treatment. After 15 days, the experiment was interrupted and the lizards were again weighed and measured as described above.

To investigate the effects of sex ratio environment on a sexually dimorphic trait, we chose relative head length as a measure because this allometry is consistently biased toward males having larger heads throughout Sauria (lizards: Olsson and Madsen, '98; Lacertids in general: Brana, '96; *L. vivipara*: Dély, '81; Smajda and Majlath, '99; Uller, unpublished data). Thus, this makes it possible to make directional predictions with respect to trait development and sex ratio environment for both sexes. To get a measure of relative head length, we used residuals from a regression of head length on snout-vent length. Before submitting our residuals for further statistical analyses, we graphically confirmed that head length is linearly (and not curvilinearly) related to SVL by plotting our data. Any other relationship would confound our analyses of sex-specific gestation environment effects. All statistical analyses were done in SAS (SAS Institute, '96).

RESULTS

Of the 56 females, 42 gave birth to 206 offspring with no difference in overall sex ratio between populations (ANOVA: $F_{5,37}=1.51$, $P=0.21$). The clutches were assigned to three different categories based on sex ratio: male, female, or nonbiased (i.e., equal numbers of males and females). As there is no resorbtion of embryos and there were no abortions, the sex ratio at parturition is equivalent to the sex ratio in utero. The mean value of relative head length was calculated for each sex within each clutch to avoid pseudoreplication. As there was no difference between the populations in relative head length (ANOVA, Females: $F_{5,36}=0.93$, $P=0.47$; Males: $F_{5,35}=0.68$, $P=0.64$), populations were pooled in the remaining analyses.

There was a highly significant interaction effect of offspring sex and prenatal sex ratio environment on relative head length (ANOVA: $F_{5,47}=6.53$,

$P=0.0001$), with males having relatively larger heads than females (Table 1). To control for the allometric trend in the mothers, we incorporated her relative head length as a covariate, but this did not change the outcome of our analysis. Separate analyses for the sexes showed that males developing in a female-biased clutch had significantly smaller heads than males developing in male-biased clutches (t-test: $t=3.14$, $P=0.0037$; Table 1; Fig. 1), whereas females developing in male-biased clutches had significantly larger heads than females developing in a female-biased clutch (t-test: $t=2.23$, $P=0.0328$; Table 1; Fig. 1). Individuals developing in clutches of equal sex ratio were intermediate in their relative head length (Table 1). There was no difference between sex ratio environments in total body mass for either sex ($P>0.40$ for both sexes).

The differences in relative head length between males from different pre-parturient sex ratio categories remained after the growth period in males, whereas the difference in females fell short of significance at the 0.05 level (t-test: $t=3.55$, $P=0.0016$, and $t=0.945$, $P=0.352$, males and females, respectively). However, the interaction between sex and sex ratio environment on relative head length was still highly significant at the end of the growth period (ANOVA: $F_{5,\ 106}=5.05$, $P=0.003$), with temperature treatment (long vs. short period of preferred body temperature) also having a significant effect ($F_{1,106}=10.52$, $P=0.0016$). Thus, the allometric relationship between head and body length changed during growth, and this effect depended on the temperature regime during the growth period. Furthermore, there was a significant interaction effect of sex and prenatal sex ratio on relative growth in head length, i.e., males and females differed in their allometric growth trajectories depending on the pre-parturient sex ratio of their clutch ($F_{5,67}=2.80$, $P=0.0235$; Fig. 1).
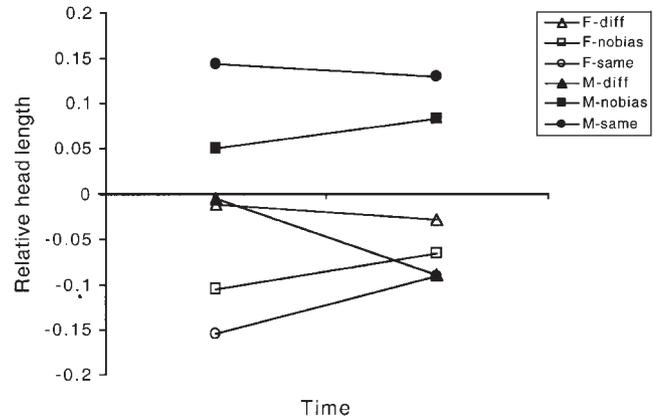
Fig. 1. Development of relative head length for both sexes and different prenatal sex ratios. The measurements were taken at parturition and after a growth period of 15 days. F=female; M=male; diff=clutches biased to opposite sex; same=clutches biased to same sex; no bias=clutches of equal sex ratio. The differences between clutches biased to opposite and same sex are statistically different at birth, with the difference remaining significant after 15 days of growth in males.

DISCUSSION

This study shows that pre-parturient sex ratios have significant sex-dependent effects on offspring allometry in natural populations of lizards. Males developing in female-biased clutches were feminized, whereas females developing in male-biased clutches were masculinized. Further support that these effects are indeed related to the sex ratio per se (or a maternal tactic relating to the clutch sex ratio) is that offspring developing in clutches of equal sex ratios were intermediate in degree of feminization/masculinization (Table 1; Fig. 1). In the present study, we had no knowledge of the intrauterine positions of developing young, but instead compared fetuses in same- and opposite-sex biased clutches with respect to characteristically sexually dimorphic traits. In reptiles, as in birds, maternally derived sex steroids can be

TABLE 1. *Relative head length $\pm$ SE for both sexes and all types of clutches[1]*

	F-diff	F-no bias	F-same	M-diff	M-no bias	M-same
Relative head	−0.0109	−0.1046	−0.1541	−0.0053	0.0502	0.1434
length at birth	±0.0548	±0.0610	±0.0335	±0.0299	±0.0548	±0.0353
Relative head	−0.0291	−0.0664	−0.0908	−0.0892	0.0832	0.1230
length after 15 days	±0.0542	±0.0568	±0.0347	±0.0653	±0.0652	±0.0283

[1]F=female; M=male; diff=clutches biased to opposite sex; same=clutches biased to same sex; no bias=clutches of equal sex ratio. The differences between clutches biased to opposite and same sex are statistically different at birth, with the difference remaining significant after 15 days of growth in males.

deposited in the yolk (e.g., Schwabl, '93; Lovern and Wade, 2001). Thus, females that give birth to mostly males could allocate more testosterone to all eggs and females with mostly daughters more oestrogen, thereby influencing offspring allometry in both sexes. Furthermore, even though sex chromosomes have been described for some *L. vivipara* populations, it is unknown whether steroid levels will also influence the probability of developing into a male or female. In the painted turtle (*Chrysemys picta*), a species with temperature-dependent sex determination (TSD), maternally derived yolk steroids have been linked to offspring sex ratio (Bowden et al., 2000), and in the leopard gecko (*Eublepharis macularius*), a lizard also exhibiting TSD, embryonic temperature influences reproductive characteristics as an adult (Gutzke and Crews, '88; Rhen and Crews, '99). In birds, it has been suggested that the steroid level in the egg could be part of the sex determining process by influencing the segregation of sex chromosomes (Petrie et al., 2001), but the generality of this hypothesis across taxa is unknown.

In reptiles, it has been suggested that oestrogen is the organizing hormone during sex differentiation and that females convert testosterone into oestrogen, catalysed by the aromatase system (Crews et al., '95; Pieau et al., '99; Osypka and Arnold, 2000). In our study, however, females developing in male-biased clutches were masculinized at parturition, suggesting that aromatisation of testosterone cannot explain our results for *L. vivipara*. To separate the fetal interaction and maternal steroid allocation hypotheses, one would have to know the position and sex of the offspring in the uterus and only further studies in which, for example, the embryos were isotope-marked or dyed could make this distinction.

The allometry effect at birth persisted over the growth period in males but not in females. Thus, the head-SVL growth trajectories after birth differed between the sexes with respect to gestation environment (Fig. 1). Males developing in male-biased clutches had relatively larger heads also after the growth period, whereas males developing in female-biased clutches showed a further decrease in body-size specific head size, making the differences between males from different sex ratio environments even more pronounced over time. In females, however, relative head size in male-biased clutches declined during the growth period and, therefore, the sex ratio environment effect disappeared. Thus, the effect of clutch sex ratio on offspring allometry differed in

strength between the sexes after parturition, suggesting that gestation environment effects (probably steroid-induced) could have longer-lasting effects in males than in females. In the closely related sand lizard (*Lacerta agilis*), both males and females with relatively larger heads have higher reproductive success (Olsson and Shine, '96). In both sexes, a large head probably increases the range of accessible prey items, resulting in higher fecundity in females, while in males large heads are also sexually selected (*L. agilis*: Olsson and Shine, '96; Lacertids in general: Brana, '96). Thus, males developing in female-biased clutches, being born with generally smaller heads, should be at a selective disadvantage compared to males developing in male biased clutches. Such a scenario is supported by data on the Mongolian gerbil, which shows intrauterine position effects on male reproductive success (Clark and Galef, '98). In gerbils, however, there may also be a testosterone-related trade-off between mate acquisition and paternal behaviour, with paternal care being compromised at high testosterone levels (Clark et al., '97). Because lizards do not exhibit paternal care, it seems likely that male common lizards gestated with mostly females are indeed at a selective disadvantage, should the diminished relative head size persist later in life.

In homeotherms, such as birds and mammals, incubation temperature is unlikely to complicate the interpretation of gestation environment effects. In ectotherms, however, it is well established that the thermal incubation environment may influence offspring shape (Shine et al., '95; Elphick and Shine, '98). Thus, maternal basking behaviour could influence the relative head size of sons and daughters. Interestingly, in the present study, males and females also differed in their head size allometry response to thermal treatment during post-parturient growth.

In conclusion, our study shows that an offspring's relative head size is influenced by the sex ratio of the clutch during gestation, and that this effect differs between sons and daughters. Males developing in female-biased clutches were feminized, and females developing in male-biased clutches were masculinized. Offspring developing from clutches of equal sex ratio were intermediate in degree of masculinization/feminization. This is the first study, to our knowledge, to demonstrate sex-specific sex ratio effects on offspring allometry in a nonmammalian species. More importantly, it suggests a model system in which not only proximate but also ultimate causation of gestation

effects on offspring phenotype could be examined in the wild with much more ease than with the mammalian models used in laboratory populations.

LITERATURE CITED

Bauwens D, Verheyen R. 1985. The timing of reproduction in the lizard *Lacerta vivipara*: differences between individual females. J Herp 19:353–364.

Bernardo J. 1996. Maternal effects in animal ecology. Am Zool 36:83–105.

Bowden RM, Ewert MA, Nelson CE. 2000. Environmental sex determination in a reptile varies seasonally and with yolk hormones. Proc R Soc Lond B Biol Sci 267:1745–1749.

Brana F. 1996. Sexual dimorphism in lacertid lizards: male head increase vs. female abdomen increase? Oikos 75:511–523.

Clark MM, Galef Jr BG. 1995. Prenatal influences on reproductive life history strategies. Trends Ecol Evol 10:151–153.

Clark MM, Galef Jr BG. 1998. Perinatal influences on the reproductive behavior of adult rodents. In: Mousseau TA, Fox CW, editors. Maternal effects as adaptations. New York: Oxford University Press. p 261–271.

Clark MM, Tucker L, Galef Jr BG. 1992. Stud males and dud males: intrauterine position effects on the reproductive success of male gerbils. Anim Behav 43:215–221.

Clark MM, Karpluk P, Galef Jr BG. 1993. Hormonally mediated inheritance of acquired characteristics in Mongolian gerbils. Nature 364:712.

Clark MM, Desousa D, Vonk J, Galef Jr BG. 1997. Parenting and potency: alternative routes to reproductive success in male Mongolian gerbils. Anim Behav 54:635–642.

Crews D, Cantu AR, Begeron JM, Rhen T. 1995. The relative effectiveness of androstenedione, testosterone, and estrone precursors to estradiol in sex reversal in the red-eared slider turtle (*Trachemys scripta*), a turtle with TSD. Gen Comp Endocrinol 100:119–127.

Dély OG. 1981. Über die morphologische variation der Zentral-Osteuropäischen Bergeidesche (*Lacerta vivipara* Jacquin). Vert Hungar 20:5–54.

Elphick M, Shine R. 1998. Long-term effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). Biol J Linn Soc 63:429–447.

Even MD, Dhar MG, vom Saal FS. 1992. Transport of steroids between fetuses via amniotic fluid in relation to the intrauterine position phenomenon in rats. J Reprod Fertil 96:709–716.

Gandelmann R, vom Saal FS, Reinisch JM. 1977. Contiguity to male fetuses affects morphology and behaviour of female mice. Nature 266:722–724.

Gutzke WHN, Crews D. 1988. Embryonic temperature determines adult sexuality in a reptile. Nature 332:832–834.

Harlow P. 1996. A harmless technique for sexing hatchling lizards. Herp Rev 2:71–72.

Holekamp KE, Smale L, Simpson HB, Holekamp NE. 1984. Hormonal influences on natal dispersal in free-living Belding's ground squirrels (*Spermophilus beldingi*). Horm Behav 18:465–483.

Lovern MB, Wade J. 2001. Maternal plasma and egg yolk testosterone concentrations during embryonic development in green anoles (*Anolis carolinensis*). Gen Comp Endocrinol 124:226–235.

Mousseau TA, Fox CW, eds. 1998. Maternal effects as adaptations. New York: Oxford University Press.

Olsson M, Madsen T. 1998. Sexual selection and sperm competition in reptiles. In: Birkhead TR, Møller AP, editors. Sperm competition and sexual selection. Cambridge: Academic Press. p 503–578.

Olsson M, Shine R. 1996. Does reproductive success increase with age or with size in species of indeterminate growth? A case study using sand lizards (*Lacerta agilis*). Oecologia 105:175–178.

Olsson M, Shine R. 1999. Timing of parturition as a maternal care tactic in an Alpine lizard species. Evolution 52:1861–1864.

Osypka N, Arnold S. 2000. The developmental effect of sex ratio on a sexually dimorphic scale count in the garter snake *Thamnophis elegans*. J Herp 34:1–5.

Panigel M. 1956. Contribution a l'etude de l'ovoviviparité chez les reptiles: gestation et parturition chez le lézard vivipare *Zootoca vivipara*. Ann Des Sc Nat Zool 11:571–668.

Petrie M, Schwabl H, Brande-Lavridsen N, Burke T. 2001. Sex differences in avian yolk hormone levels. Nature 412:498–499.

Pieau C, Dorizzi M, Richard-Mercier N. 1999. Temperature-dependent sex determination and gonadal differentiation in reptiles. Cell Mol Life Sci 55:887–900.

Rhen T, Crews D. 1999. Embryonic temperature and gonadal sex organize male-typical sexual and aggressive behavior in a lizard with temperature-dependent sex determination. Endocrinology 140:4501–4508.

Schwabl H. 1993. Yolk is a source of maternal testosterone for developing birds. Proc Natl Acad Sci U S A 90:11446–11450.

Schwabl H. 1996. Maternal testosterone in the avian egg enhances postnatal growth. Comp Biochem Physiol A Physiol 114:271–276.

Shine R, Elphick M, Harlow P. 1995 Sisters like it hot. Nature 378:451–452.

Sinervo B. 1990. The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. Evolution 44:279–294.

Smajda B, Majlath I. 1999. Variability of some morphological traits of the common lizard (*Lacerta vivipara*) in Slovakia. Biologia 54:585–589.

Van Damme R, Bauwens D, Verheyen R. 1987. Thermoregulatory responses to environmental seasonality by the lizard *Lacerta vivipara*. Herpetologica 43:405–415.

Vandenbergh JG, Huggett CL. 1994. Mother's prior intrauterine position affects the sex ratio of her offspring in house mice. Proc Natl Acad Sci U S A 91:11055–11059.

Via S. 1993. Adaptive phenotypic plasticity: target or by-product of selection in a variable environment? Am Nat 142:352–365.

vom Saal FS. 1989. Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses in utero. J Anim Sci 67:1824–1840.

Yaron Z. 1985. Reptilian placentation and gestation: structure, function, and endocrine control. In: Gans C, Billet F, editors. Biology of the reptilia, volume 15, Development B. New York: John Wiley & Sons. p 527–604.