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Maternal basking behaviour determines offspring sex in a viviparous reptile

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Two primary dichotomies within vertebrate life histories involve reproductive mode (oviparity versus viviparity) and sex determination (genotypic sex determination versus environmental sex determination). Although reptiles show multiple evolutionary transitions in both parameters, the co-occurrence of viviparity and environmental-dependent sex determination have heretofore been regarded as incompatible. Our studies on the viviparous lizard *Niveoscincus ocellatus* show that the extent of basking by a female influences the sex of her offspring. Critically, our data reveal this effect both in the field (via correlations between date of birth and litter sex ratio) and in a laboratory experiment (females with reduced basking opportunities produced more male offspring). Changes in thermoregulatory behaviour thus allow pregnant female lizards to modify the sex of their offspring.

Keywords: environmental sex determination; sex ratio; squamate; viviparity

1. INTRODUCTION

Two major axes of vertebrate life history involve reproductive mode (oviparity versus viviparity) and sex determination (genotypic sex determination (GSD) versus environmental sex determination (ESD)). Both traits show multiple independent evolutionary origins within the Reptilia, making this an ideal group in which to investigate evolutionary processes during such transitions (Bull 1980; Shine 1999). Both ESD (usually temperature-dependent sex determination (TSD)) and GSD occur in oviparous (egg-laying) reptiles, but the consensus has been that viviparity (live-bearing) is incompatible with TSD. The rationale for this claim is straightforward: because behavioural thermoregulation results in relatively high, stable maternal (and thus embryonic) temperatures, any thermally dependent system for determining offspring sex would yield highly skewed (and thus evolutionarily disadvantageous) offspring sex ratios (Bull 1980).

Robert & Thompson (2001) recently reported, however, that TSD does indeed occur in a viviparous lizard. Robert & Thompson's (2001) report that female lizards (*Eulamprus tympanum*) maintained in the laboratory at high and constant temperatures produced exclusively male offspring posed a strong challenge to prevailing paradigms that viewed viviparity and TSD as incompatible. Do such sex-allocation shifts occur in nature, where females select their own diurnal thermal regimes? Robert and Thompson's study was unclear. First, females were held at (biologically unrealistic) constant temperatures, eliminating opportunities for thermoregulatory behaviour. Second, their litters from field-collected females did not show sex-ratio skewing (Robert & Thompson 2001). To understand the adaptive significance of any link between maternal basking and sex allocation we must mimic natural situations as closely as possible. Field data are essential, but can reveal correlations only. To establish causal links between maternal basking and offspring sex, we need experimental studies that manipulate basking opportunities (not maternal temperature *per se*) to examine the effects on offspring sex. Here, we describe a study combining field and laboratory investigations on sex allocation in a viviparous skink that belongs to a lineage that is phylogenetically distant (Honda *et al.* 2000) from the species studied by Robert & Thompson (2001), and that thus represents an independent origin of viviparity.

2. MATERIAL AND METHODS

(a) *Study species and analyses of sex ratios*

Niveoscincus ocellatus are small viviparous scincid lizards occurring over a wide geographical range in Tasmania, Australia (Wapstra 2000). Ovulation and fertilization occur early in spring (September), with parturition following a long weather-dependent gestation approximately 3.5 months later (Wapstra *et al.* 1999). We examined sex allocation in a coastal population (42°34′ S, 147°52′ E) both in the field and in the laboratory.

We conducted two analyses of sex ratio: at the levels of the population and of the individual litter. We used a binomial test to examine population-level effects (i.e. deviation from a predicted null of 50% male). Ideally, offspring sex would be further modelled using binary logistic regression where the model included maternal identity, birth date and experimental regime (Wilson & Hardy 2002). However, the small litter sizes (typically two to three) precluded this approach owing to extensive quasi-complete separation of data points. Rather, we used logistic regression to model *litter* sex ratios.

(b) *Sex allocation in the field*

In this cool-climate population, the timing of ovulation is highly synchronized among females but gestation length reflects maternal basking opportunity: mean birth dates are later in cooler years (Wapstra *et al.* 1999). If TSD effects occur in *N. ocellatus*, field data should reveal a correlation between parturition date and litter sex ratio because gestation length reflects mean maternal body temperature. To test this prediction, we captured females ($n = 74$) late in pregnancy. They were maintained in the laboratory individually under standard conditions (14 L : 10 D; 12 h access to basking lights) until parturition. At birth, we recorded standard female and offspring characteristics, including offspring sex.

(c) *Experimental manipulation of basking opportunities*

If *N. ocellatus* displays TSD, manipulating female basking opportunities should modify the sex ratios of their offspring. Females were captured soon after ovulation (confirmed by dissection in a subsample) and divided randomly into two treatments: either 4 h ($n = 18$) or 10 h ($n = 14$) daily access to heat-lamps, during which time they could maintain their preferred body temperature (*ca.* 30 °C). Outside of these times, the lizards exhibited body temperatures close to ambient (14 °C). This experimental design mimics spatial and temporal heterogeneity in weather conditions and, thus, basking opportunities in the lizards' natural habitat (Wapstra 2000). All females (except one with access to 10 h basking from which no birth was recorded) gave birth within the dates recorded in the field (Wapstra *et al.* 1999). At birth, we measured standard offspring characters (snout vent length (SVL), total length, mass) and determined their sex by eversion of hemipenes.

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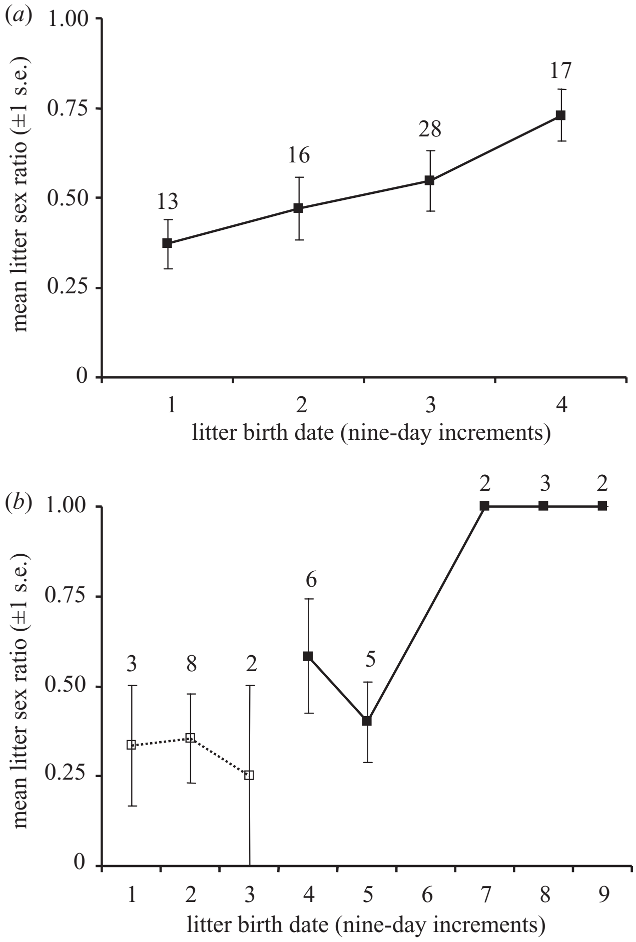

Figure 1. Relationship between birth date (in nine-day increments since 1 January) and mean litter sex ratio for *Niveoscincus ocellatus* from (*a*) litters produced by females from a natural population; and (*b*) litters produced by pregnant females given the opportunity to bask for either 4 h d^{-1} (filled squares and solid line) or 10 h d^{-1} (open squares and dotted line). Sample sizes are shown above the standard error bars.

3. RESULTS

(a) *Patterns of sex allocation in the field*

All females gave birth within three weeks of capture and thus completed more than 90% of their gestation in the field. The median birth date was 19 January (range 1 January–5 February). Female body size was significantly correlated with reproductive output (Pearson's correlation coefficient = 0.63, $p < 0.0001$) and litter size (range of 1–4; Spearman's correlation coefficient = 0.46, $p < 0.0001$), but not with relative clutch mass (RCM) (combined mass of offspring/female post-partum mass) or offspring size. The 'population' sex ratio did not deviate from a null of 50% male (96 males; 78 females, binomial test $p = 0.20$; 174 offspring from 74 females). However, logistic regression revealed that litter sex ratio varied significantly with birth date: male-biased litters were produced later in the season ($r^2_{\text{model}} = 0.0870$, Wald χ^2 for birth date = 5.6319, $p = 0.0176$; $r^2_{\text{model}} = 0.1870$, Wald χ^2 for birth date = 13.6371, $p = 0.0002$ with litter size as weighting factor; figure 1*a*). Litter sex ratio was not significantly related to female size, RCM or fat stores (all $p > 0.05$).

(b) *Experimental manipulation of basking opportunities*

At the beginning of the experiment, females in the two treatment groups were similar in all respects (SVL, mass, predicted litter size based on palpation of recently ovulated follicles, all $p > 0.05$). Basking opportunity significantly affected birth date and offspring phenotype, including their mass and SVL (table 1). Females with limited thermoregulatory opportunities produced predominantly male offspring (69% male: 31 males; 14 females (one offspring not sexed), binomial test $p = 0.016$ against null of 50% male). By contrast, females with greater access to basking opportunities produced predominantly female offspring (67% females: nine male; 18 females, binomial test $p = 0.12$ against a null of 50% male). Thus, sex ratios differed significantly between groups ($G = 8.75$, $p < 0.0015$). Logistic modelling of litter sex ratios revealed a significant relationship between birth date and litter sex ratio, with basking treatment explaining no further variation in litter sex ratio ($r^2_{\text{model}} = 0.41$, Wald χ^2 for birth date = 6.99, $p = 0.008$; Wald χ^2 for basking treatment = 0.47, $p > 0.5$; $r^2_{\text{model}} = 0.74$, Wald χ^2 for birth date = 20.66, $p < 0.0001$; Wald χ^2 for basking treatment = 2.68, $p > 0.1$ with litter size as a weighting factor; figure 1*b*). Within each treatment group, male-dominated litters were more common late in the birth period within the reduced basking group (adjusted $r^2_{\text{model}} = 0.48$, Wald χ^2 for birth date = 6.01, $p = 0.014$; adjusted $r^2_{\text{model}} = 0.84$, Wald χ^2 for birth date = 17.48, $p < 0.0001$ with litter size as a weighting factor) but no such pattern was evident within the extended basking group.

4. DISCUSSION

Female viviparous lizards *N. ocellatus* can influence the sex of their offspring by modifying the amount of time spent basking during pregnancy. These results thus support the laboratory study by Robert & Thompson (2001) of TSD in another viviparous reptile. However, in contrast to their study, our results in *N. ocellatus* extrapolate to the field because (i) sex ratios of offspring born to field-collected females covaried with birth date; and (ii) offspring sex in the laboratory also covaried with birth date, achieved through experimental manipulation of female basking duration that closely mimicked conditions that varied temporally and spatially in the field.

Our results also differ from those of Robert & Thompson (2001) in both the direction and the degree of sex-ratio skewing induced by high maternal temperatures. First, higher maternal temperatures generated fewer sons in *N. ocellatus* but more sons in *E. tympanum*. This divergence raises the question of the adaptive significance of maternal control over offspring sex ratio. Mathematical models predict that natural selection will stabilize population sex ratios to 1 : 1 (Fisher 1930), as was the case for overall offspring sex ratios in our natural population. However, adaptive shifts in individual female allocation patterns are now well established (Trivers & Willard 1973), including in a congeneric species of snow skink (Olsson & Shine 2001). The benefit of facultative sex-ratio adjustment may relate to sex differences in the importance of adult body size for reproductive success. Reproductive output of females depends on maternal body length (Wapstra 2000; this study), whereas reproductive success

Table 1. Effect of female basking opportunity on birth date and offspring characteristics of snow skinks in a laboratory experiment. (See Wapstra (2000) for full description of effects on female and offspring characteristics: offspring sex ratios have not been previously reported.)

trait	extended female basking opportunity (10 h d^{-1})	reduced female basking opportunity (4 h d^{-1})	statistical result
birth date	1 January–27 January (median: 10 January)	29 January–19 March (median: 11 February)	$F_{1,30} = 46.52$, $p < 0.0001$
offspring mass (mg)	541.2 ± 9.48	475.5 ± 10.30	$F_{1,30} = 20.38$, $p < 0.0001$
offspring SVL (mm)	29.8 ± 0.23	29.11 ± 0.20	$F_{1,30} = 4.27$, $p < 0.05$
offspring sex ratios	9 male : 18 female	31 male : 14 female	see § 3

may be less tightly linked to body size in adult males (Olsson *et al.* 2002). Because birth date influences adult body size, the disadvantages of late birth may be greater for daughters than for sons. This might explain the opposite direction of the temperature effect in *E. tympanum*. Higher-temperature incubation (i.e. earlier birth) generated more sons (Robert & Thompson 2001), and larger males have higher reproductive success in at least one *Eulamprus* species (Morrison *et al.* 2002). Under this scenario, female *Eulamprus* that give birth late in the season overproduce the sex (daughters) that experiences a lesser disadvantage from later birth. These ideas remain speculative, but make falsifiable predictions amenable to robust testing in field studies.

Our results further differ in the magnitude of the effect reported: Robert & Thompson (2001) reported dramatic sex-ratio shifts, with 100% male litters from some treatments. We found more subtle shifts in *N. ocellatus*, as might be expected for the following three reasons. First, variation among females in mean body temperatures experienced during gestation will be continuous rather than dichotomous. Thus, parturition dates and litter sex ratios are also likely to be distributed continuously, not dichotomously. Second, steep norms of reaction linking sex ratios to female body temperatures are unlikely to be evolutionarily stable because minor seasonal climatic fluctuations would alter population-wide sex ratios, particularly in relatively short-lived species (Bull & Bulmer 1989). Third, other factors have the potential to alter sex allocation in concert with, or in opposition to, maternal thermal profiles (see Olsson & Shine 2001).

Although we do not understand the physiological mechanism(s) by which female *N. ocellatus* (or other viviparous reptiles) control the sex of their offspring, we can rule out mechanisms that operate prior to ovulation (e.g. sperm choice by females (Olsson & Shine 2001; Komdeur *et al.* 2002)) because the females in our laboratory experiment had ovulated (and their eggs were already fertilized) before the experimental treatment was applied. Similarly, we can rule out differential sex-specific mortality of embryos (Burger & Zappalorti 1988) because the number of ovulated eggs (based on palpation) was identical to the number of offspring produced. Recent work on the congeneric *N. microlepidotus* suggests a plausible mechanism to link maternal temperature to phenotypic sex. J. Girling (personal communication) found that testosterone production by embryonic gonads is highest when embryos develop at low temperatures (i.e. those experienced by embryos if females are unable to bask). Presumably, this thermal mechanism is somehow superimposed on an ancestral GSD system. For example, the oviparous skink, *Bassiana duperryi*, has clear heteromorphic sex chromosomes, but incubation temperatures can override sex chromosomes to determine sex (Shine *et al.* 2002). Snow skinks (*Niveoscincus*) lack overt sex chromosomes, but related taxa have XY heterogamety and genetic control of sex determination is ancestral within the lineage (Donellan 1985).

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