Climate effects on offspring sex ratio in a viviparous lizard

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Summary

1. Understanding individual and population responses to climate change is emerging as an important challenge. Because many phenotypic traits are sensitive to environmental conditions, directional climate change could significantly alter trait distribution within populations and may generate an evolutionary response.

2. In species with environment-dependent sex determination, climate change may lead to skewed sex ratios at hatching or birth. However, there are virtually no empirical data on the putative link between climatic parameters and sex ratios from natural populations.

3. We monitored a natural population of viviparous lizards with temperature-dependent sex determination (Niveoscincus ocellatus) over seven field seasons. Sex ratios at birth fluctuated significantly among years and closely tracked thermal conditions in the field, with the proportion of male offspring increasing in colder years.

4. This is the first study to demonstrate the effect of local climatic conditions (e.g. temperature) on offspring sex ratio fluctuations in a free-living population of a viviparous ectotherm. A succession of warmer-than-usual years (as predicted under many climate-change scenarios) likely would generate female-biased sex ratios at birth, while an increase in interannual variation (as also predicted under climate change scenarios) could lead to significant fluctuations in cohort sex ratios. If cohort sex ratio bias at birth leads to adult sex ratio bias, long-term directional changes in thermal conditions may have important effects on population dynamics in this species.

Key-words: climate change, climate variability, environmental sex determination, reptile, squamate

Introduction

Climatic conditions can influence population dynamics via several pathways, including by modifying the strength of sexual selection (Møller 2004; Blanckenhorn et al. 2006), reproductive output (Winkler, Dunn & McCulloch 2002; Dunn 2004; Angilletta, Oufréro & Leaché 2006; Chamaille-Jammes et al. 2006), and survival (Angilletta et al. 2004; Grosbois et al. 2006; Hance et al. 2007). The extent to which directional or fluctuating changes in climatic conditions affect individual performance and, consequently, induce a population-level response depends not only on the direct effects of environmental conditions (e.g. thermal conditions may affect food availability and, therefore reproductive output: Dunn 2004), but also more complex pathways such as reliance on environmental cues for adaptive phenotypic plasticity and the timing of life-history events (Pulido & Berthold 2004). For example, increased temperatures over recent decades have generated a mismatch between food availability and the onset of breeding in some bird populations, reducing the survival of chicks and, thus, population size (Visser, Both & Lambrechts 2004; Both et al. 2006; but see Charmantier et al. 2008).

Although most research on the link between climatic variation and population viability has focused on the effects of climate on offspring number (e.g. Dunn 2004; Chamaille-Jammes et al. 2006), environmental variation also can affect phenotypic traits of offspring (Lourdais et al. 2004). For example, sex determination in many reptiles is sensitive to the
thermal regimes experienced during embryonic development (Janzen & Paukstis 1991; Shine 1999; Robert & Thompson 2001; Valenzuela & Lance 2004; Wapstra et al. 2004). Consequently, natural variation in nest temperatures (in oviparous species) or maternal basking opportunities (in viviparous species) may affect offspring sex ratios. Any local or annual biases in cohort sex ratios resulting from such effects ultimately may select against temperature-dependent sex determination (TSD), particularly in short-lived species (Bulmer & Bull 1982; Van Dooren & Leiner 2003; Girondot et al. 2004). In extreme cases, local populations may go extinct and there is a serious concern that climate change may lead to population declines of many reptiles with TSD (Janzen 1994; Robert & Thompson 2001; Morjan 2003a; Janzen & Phillips 2006). However, studies of natural variation in sex ratios in relation to geographical and annual variation in thermal conditions are rare (Janzen 1994; Doody et al. 2006; Janzen & Phillips 2006).

One potential adaptive response to negative effects of climatic variation on offspring sex ratio is for females to minimize the variation in sex ratios via active nest site choice. In the water dragon, Physignathus lesueurii, maternal nest site choice (selecting open vs. closed nest sites) eliminates geographical variation in nest temperatures (Doody et al. 2006). However, facultative adjustment of offspring developmental conditions will be constrained by the range of environmental conditions that allow for successful embryonic development, and by trade-offs with other traits in both offspring and parents (see Morjan 2003b and references therein). For example, active nest site choice is only a viable strategy to the degree that suitable conditions for embryos are achievable. Even if thermally suitable sites can be found, covariation with soil moisture levels may render such sites unsuitable for developing eggs. Similarly, in viviparous lizards where offspring sex is affected by maternal basking opportunity (Robert & Thompson 2001; Wapstra et al. 2004), increased basking in thermally challenging environments may compromise female survival via increased exposure to predators (e.g. Huey & Slatkin 1976; Shine 1980; Downes 2001, see also Spencer & Thompson 2003) and, therefore, be selected against. Selection on hatching or birth date also will compromise an evolutionary response towards selection of lower nest temperatures or reducing basking. Thus, compensatory strategies are unlikely to completely eliminate climatic effects on offspring sex ratio in TSD species.

Here we use a long-term field data set to examine if fluctuations in population-wide offspring sex ratios correlate with environmental thermal conditions in a viviparous skink (Niveoscincus ocellatus Gray 1845), a species in which maternal basking behaviour affects offspring sex (Wapstra et al. 2004). If climatic conditions vary from year to year, and females are unable to flexibly adjust their thermoregulatory behaviour sufficiently to buffer that variation, the thermal sensitivity of sex ratios in this species thus may lead to annual variation in offspring sex ratios, with potentially important consequences for population dynamics (Girondot et al. 2004).

**Materials and methods**

**STUDY SPECIES AND LIZARD COLLECTION**

*N. ocellatus* are small viviparous scincid lizards that occur over a wide geographical range in Tasmania, Australia (Wapstra 2000; Wapstra et al. 2004). The biology of this species has been described previously (e.g. Wapstra et al. 1999, 2004; Wapstra 2000) and only relevant details are repeated here. In short, we studied a population of individually marked lizards on the east coast of Tasmania (42°34’S, 147°52’E). At this site, all females reproduce annually producing a litter of one to four offspring (Wapstra & Swain 2001). Individuals mature relatively early at 2–3 years of age and live for up to 7 years (Wapstra, Swain & O’Reilly 2001). The timing of ovulation is highly synchronized among females and is consistent between years (assumed here to be 1 October in each year as in Wapstra 2000; Wapstra et al. 2004). Gestation is long (3–4 months) and variable among years, reflecting annual variation in maternal basking opportunity with birth occurring later in cooler years (Wapstra et al. 1999; Wapstra 2000). Experimental work has confirmed that maternal basking behaviour influences offspring sex ratio; females given access to greater basking opportunities during gestation produce female-biased sex ratios among their offspring, whereas females restricted to lesser basking opportunities produce primarily sons rather than daughters (Wapstra et al. 2004). Gonadal differentiation in embryonic *N. ocellatus* occurs in the first half of gestation (Neaves et al. 2006) as is typical in squamates (Shine, Warner & Radder 2007 and references therein).

Since the Austral summer of 2000/01, we have followed a standard field and laboratory protocol that involves field capture of female skinks at the end of gestation (late December, past the thermal sensitive period of sex determination, Wapstra et al. 1999) by mealworm fishing or noosing. Upon capture, females are identified individually using unique toe clips (or a new one is assigned) and their position recorded to ±5 m within the study site using a handheld global positioning system (GPS). Females are returned to a temperature-controlled laboratory (ambient temperature 16°C ± 1°C) at the University of Tasmania where they are held in plastic terraria (30 × 20 × 20 cm) each containing cover and a basking light to provide a thermal gradient for basking from 16°C to 40°C. Water is available *ad libitum* and lizards are fed three times per week on live insects and crushed fruit. Terraria are checked twice daily for neonates. At birth, offspring are measured (mass, ±0.1 mg, snout-vent length, ±0.01 mm) and sexed by hemipene eversion (Wapstra et al. 2004). Sizes of adult females are similarly recorded (mass, ±0.1 mg, snout-vent length, ±1 mm). All offspring are permanently marked before they and their mothers are released at the original site of capture.

**CLIMATIC DATA**

Meteorological data were obtained from a Bureau of Meteorology station situated < 5 km from our study site (42°55’S, 147°87’E). As a measure of the thermal conditions (basking opportunity) experienced by individual female skinks while gravid in the field, we used the mean of daily maximum temperatures experienced (i) during the thermally sensitive phase of gestation (first half of gestation, here assigned as 1 October–14 Nov based on averages across years), and (ii) after the thermally sensitive period of gestation (15 November–31 December) (Wapstra 2000; Wapstra et al. 2004; Neaves et al. 2006).
STATISTICAL ANALYSES

Over the 7 years of our study, we collected 682 females that gave birth to 1567 offspring in the laboratory. Of these females, 227 were collected in 2 or more years; we therefore used female identity as a random repeated factor in our analyses where appropriate (Uller et al. 2006). Sex-ratio variation across the 7 years of study was analysed in four ways. First, we analysed interannual variation in litter sex-ratios (proportion of males in a litter) in a generalized linear mixed model (GLMM) using PROC GLIMMIX in SAS version 9.2 with a binomial distribution and a logit link function (Littell et al. 1996). Female identity was entered as a random repeated factor, while year of collection was entered as a fixed factor and clutch birthdate and female body size were included as covariates (N = 680 clutches). Second, we analysed whether cohort sex ratio deviated for any given year from the equal (50:50) proportion expected from theoretical models (e.g. Fisher 1930) using Pearson’s chi-squared analyses on frequencies of male and female offspring. Third, we analysed interannual variation in litter sex ratios as a function of gestation temperatures with a GLMM using the GLIMMIX macro in SAS, again with a binomial distribution and a logit link function. Female identity was entered as a random repeated factor, and first and second half gestation temperatures were entered as fixed factors (N = 682 clutches). For both GLMM models, we were explicitly interested in testing the contributions of fixed effects and covariates above and beyond that already accounted for by repeat females (Krackow & Tadadle 2001). Therefore, we report estimates for all fixed effects (higher-order interactions were all nonsignificant) where each model always included the random effect of female identity. Significance of fixed factors was tested using F-tests, with the degrees of freedom calculated using Satterthwaite’s approximation (Littell et al. 1996). Significance of random effects was tested using chi-squared test comparing the full final model with its appropriate restricted version (i.e. dropping female identity and comparing the fits of the models). Critical values of chi-squared used to test significance of model log-likelihood differences were \( \chi^2_{(105)} = 3.84 \) and \( \chi^2_{(100)} = 6.63 \) (Self & Liang 1987). Finally, we used linear regression with cohort sex ratio as the dependent variable and mean maximum temperatures during the first and second half of gestation in each year to test for a correlation between temperature and cohort offspring sex ratio at parturition and additionally we tested for a link between cohort offspring sex ratio and mean birth date within the population (N = 7 years for both models).

Results

Litter sex-ratios of *N. ocellatus* at our study site fluctuated significantly across the 7 years of study \( [F_{6,621} = 5.89, P < 0.0001] \); with the mean sex ratio of litters in a year varying from male dominated (mean litter sex ratio 0·67) in 2003/04 to female dominated (mean litter sex ratio 0·45) in 2005/06. This interannual variation was not explained by variation in clutch birth date \( [F_{6,621} = 2.73, P = 0.098] \), female body size \( [F_{6,580} = 0.26, P = 0.61] \), or female identity \( [\chi^2_{(1)} = 2.60, P > 0.05] \). Chi-squared tests for cohort sex ratio at birth showed significant, or borderline significant, deviation from 50:50 in 5 out of 7 years \( [2000/01: \chi^2_{(1)} = 1.48, P = 0.22; 2001/02: \chi^2_{(1)} = 8.14, P = 0.004; 2002/03: \chi^2_{(1)} = 3.05, P = 0.081; 2003/04: \chi^2_{(1)} = 23.11, P < 0.001; 2004/05: \chi^2_{(1)} = 7.34, P = 0.007; 2005/06: \chi^2_{(1)} = 3.19, P = 0.074; 2006/07: \chi^2_{(1)} = 0.72, P = 0.78] \) (see Fig. 1). Low mean maximum temperatures in the field during the sensitive period of sex determination led to male-biased litter sex ratios \( [\chi^2_{(1)} = 21.81, P < 0.001] \), whereas field temperatures later in gestation did not significantly predict litter sex ratios \( [\chi^2_{(1)} = 0.01, P = 0.91] \). Inclusion of female identity in gestation temperature mixed models did not alter overall model fit \( [\chi^2_{(1)} = 2.90, P > 0.05] \). Overall, the probability of a female producing a male-biased clutch almost doubled (log odds-ratio for temperature over first half of gestation: –0.56) with a one-degree decrease in mean temperatures over the first half of gestation. Importantly, this result also held true when examining cohort sex ratio across years \( [F_{1,6} = 14.14, R^2 = 0.738, P = 0.013; \text{Fig. 2a}] \). As for the full model above, the mean maximum temperature experienced during the second half of gestation did not significantly affect offspring sex \( [F_{1,6} = 0.06, R^2 = 0.012, P = 0.815] \). Because rates of embryonic development are also temperature dependent (over the full duration of gestation), we also tested if among-year shifts in offspring sex ratios were linked to shifts in birth dates in corresponding years (see Fig. 1a). This was the case \( [F_{1,6} = 9.87, R^2 = 0.67, P = 0.024] \) with more male offspring produced in years where birth dates were delayed and more female offspring in years with earlier birth dates (Fig. 2b) suggesting a link between these thermally dependent processes.

Discussion

In species where offspring sex is sensitive to environmental conditions experienced during development, annual variation in these conditions could lead to fluctuations in sex ratios at birth or hatching. If cohort sex ratio links to adult sex ratio as we might expect in a short-lived species, such sex ratio fluctuations might lead to local or global extinction of species (Morjan 2003a) and are of serious concern for species with temperature-dependent sex determination under ongoing climate change (Janzen 1994; Robert & Thompson 2001). Here we demonstrate significant among-year variation in offspring sex ratios in the viviparous skink, *N. ocellatus*, which correlated positively with the mean maximum temperature in the field during the thermally sensitive period of gestation. Thus, natural variation in thermal conditions generated annual variation in sex ratios at birth in this species, suggesting that adult sex ratios in this population could become increasingly biased under directional climate change.

Thirteen years ago, Janzen (1994) showed that climatic conditions could drive annual variation in offspring sex ratios in a turtle species with TSD, and warned of the potential for local extinctions. Although identified as a high priority for research (e.g. Janzen & Phillips 2006), the logistical difficulties of monitoring population-level sex ratio responses to climatic variation have precluded further examples of an empirical link between climatic conditions and offspring sex ratio in other TSD species. Our results constitute the first substantial evidence that thermal conditions in the field directly affect offspring sex ratio in a lizard with thermally sensitive sexual differentiation. Importantly, many TSD lizards (including *N. ocellatus*) are considerably shorter-lived than most turtles,
suggesting that annual fluctuations in secondary sex ratios (i.e. at parturition or hatching) would have a stronger (and potentially more rapid) impact on adult sex ratios and, consequently, on population demography than envisioned by Janzen (1994, see also Bull & Bulmer 1989). However, sex-ratio fluctuations in our study species are more modest than in many turtles, which may reduce detrimental effects at the population level.

The selective forces that favoured the evolution (or maintenance) of thermally sensitive sex determination in *N. ocellatus* are unclear at this stage. However, if the more extreme sex-ratio shifts that are predicted under climate change are selected against, they might be reduced by both short- and long-term responses. First, it is possible that females could alter their behaviour on a seasonal basis (e.g. reducing their basking during warm years and increasing their basking during cold years). However, the clear covariation in both birth dates and sex ratios among years, linked to thermal conditions, indicate that such compensation (if it occurs at all) only partially reduces the direct effects of thermal variation.

Second, variation in secondary sex ratios may be reduced by facultative sex ratio adjustment in response to the operational sex ratio, in our species perhaps via adjustment of maternal basking behaviour (Olsson & Shine 2001; but see Le Galliard *et al.* 2005; Allsop *et al.* 2006; Warner & Shine 2007). However, the evolution of adaptive sex ratio adjustment in relation to population sex ratios can occur only under restricted conditions (when adult sex ratios are indicative of mating opportunities

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**Fig. 1.** Annual variation in (a) birth dates and (b) cohort offspring sex ratio of the scincid lizard *Niveoscincus ocellatus* from the seasons 2000/01–2006/07.
for offspring; Werren & Charnov 1978; Werren & Taylor 1984; West & Godfray 1997) and we doubt whether these conditions apply to our study species.

Third, increased temperatures may be associated with shifts in the timing of reproductive events that counter thermal effects on reproductive parameters, including the sex ratio (Visser et al. 2004). However, evidence for such compensatory shifts is yet to be demonstrated (see also Morjan 2003a for theoretical arguments against rapid female compensatory mechanisms) and evolutionary response to climate change may in fact be constrained in some systems by antagonistic selective pressures (Both & Visser 2001). For example, in our system, potential female compensatory shifts for variation in thermal conditions are a result of past selection to increase basking behaviour to promote rapid gestation and optimal offspring phenotype (Schwarzkopf & Shine 1991; Wapstra 2000) and balancing selection to decrease basking while gravid to reduce the associated risk of predation and loss of foraging opportunity (Shine 1980; Wapstra & O’Reilly 2001).

Fourth, on an evolutionary time-scale, large fluctuations in sex ratios may select for insensitivity of embryos to thermal conditions and, therefore, loss of TSD (Bulmer & Bull 1982; Van Dooren & Leimer 2003; reviewed in Uller et al. 2007; but see Freedberg & Taylor 2007). An evolutionary response to compensate for climate change may also occur via an increase or decrease in the pivotal (threshold) temperatures for sex determination (Ewert, Etchberger & Nelson 2004) or consistent (as opposed to flexible, see above) changes in maternal basking behaviour or preferred body temperatures (Janzen 1994; Roosenburg 1996; Morjan 2003a). However, contrary to theory (Morjan 2003a), the few empirical studies that exist suggest that pivotal temperatures are relatively fixed across climatic conditions (Ewert et al. 2004; Doody et al. 2006). Evolutionary changes in sex ratios therefore may evolve primarily via direct or indirect selection on nest site choice (in oviparous species), a highly consistent (repeatable) trait among females in several species of turtles (e.g. Bull, Vogt & Bulmer 1982; Janzen & Morjan 2001; Van Dooren & Janzen 2001; Kamel & Mrosovsky 2004; St. Juliana, Bowden & Janzen 2004) or basking behaviour in viviparous species (but see earlier arguments for constraints on this trait). Nevertheless, in both oviparous and viviparous species with TSD, short- or long-term compensation of nest or maternal temperatures can occur only over a limited thermal range that allows for successful embryonic development. Consequently, there is a need for long-term monitoring of populations to establish whether they show patterns that track (directional or fluctuating) environmental variation. Furthermore, experimental approaches (such as those employed previously with this species; Wapstra et al. 2004) are required to address the extent of intra- and interpopulational variation in sex-determining mechanisms both for parental traits (e.g. criteria for nest site choice or basking behaviour) and offspring traits (e.g., thermal threshold for sex determination) to facilitate the development of models that can predict the evolutionary response to selection (Uller et al. 2007).

Finally, we point to an intriguing possible consequence of the patterns that we have documented for population viability. We showed that cool thermal conditions lead to more male offspring and warm thermal conditions lead to proportionally more female offspring both in the field study reported here and previously in laboratory experiments (Wapstra et al. 2004). Many theorists (see Rankin & Kokko 2007 and references therein) have speculated that rates of population growth depend primarily upon the number of females, especially in a species like *N. ocellatus* where a single adult male can mate with many females (Jones, Wapstra & Swain 1997; Wapstra et al. 1999). Hence, if the cohort offspring sex ratio translates to adult sex ratio, directional climate warming could lead to enhanced population viability, at least in the short term (but see Milner-Gulland et al. 2003 and Bessa-Gomes, Legendre & Clobert 2004 for potential of population collapse after male rarity under a range of mating systems). Thus, global warming may actually enhance the viability of *N. ocellatus* populations by this higher-level demographic shift, while presumably challenging those same populations by a variety of other processes (changes in vegetation associations, food availability, presence of parasites or predators, etc.). Thus, a full understanding of the ways in which global climate change
impacts thermally sensitive species will need to take into account how individual phenotypic plasticity leads to changes in population size and demography.

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