LETTER

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Climate-driven population divergence in sex-determining systems

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Sex determination is a fundamental biological process, yet its mechanisms are remarkably diverse[1,2]. In vertebrates, sex can be determined by inherited genetic factors or by the temperature experienced during embryonic development[2,3]. However, the evolutionary causes of this diversity remain unknown. Here we show that live-bearing lizards at different climatic extremes of the species' distribution differ in their sex-determining mechanisms, with temperature-dependent sex determination in lowlands and genotypic sex determination in highlands. A theoretical model parameterized with field data accurately predicts this divergence in sex-determining systems and the consequence thereof for variation in cohort sex ratios among years. Furthermore, we show that divergent natural selection on sex determination across altitudes is caused by climatic effects on lizard life history and variation in the magnitude of between-year temperature fluctuations. Our results establish an adaptive explanation for intra-specific divergence in sex-determining systems driven by phenotypic plasticity and ecological selection, thereby providing a unifying framework for integrating the developmental, ecological and evolutionary basis for variation in vertebrate sex determination.

Vertebrates exhibit both genotypic (GSD) and temperature-dependent sex determination (TSD)[1,2]. The latter is particularly common in reptiles and both systems can co-occur within taxonomic families[3]. In addition, some species show elements of both genotypic and environmental sex determination within populations[4,5]. The causes of repeated evolutionary shifts between GSD and TSD and the origin and maintenance of mixed systems are two of the greatest unsolved problems in sex determination research[1–8]. The main reasons that diversity in reptilian sex determination has remained an enigma has been a failure empirically to link incubation temperature to ecological conditions promoting TSD and to establish theoretically that those conditions are sufficient to drive evolutionary shifts in sex-determining systems[3,7]. Here we provide both kinds of support using evolutionary models parameterized with field data to show how climatic effects on lizard life history generate evolutionary divergence in sex-determining systems via natural selection on sex ratios.

Environment-dependent sex determination can be favoured over genotypic sex determination when there are sex-specific fitness effects of environmental conditions experienced during or after the sex-determining period[9]. Temperature has a strong effect on the rate of embryonic development in ectotherm animals, with relatively cool conditions resulting in delayed birth or hatching. Sex differences in the fitness consequences of timing of birth could therefore favour integration of temperature-dependent developmental processes and gonad differentiation to ensure a match between offspring sex and birth date[10,11]. As a result, spatial or temporal variation in the strength of sex-specific selection on birth date, and therefore on TSD, may explain rapid evolutionary divergence in sex determination between populations or species[10–12].

The snow skink, *Niveoscincus ocellatus,* is a small live-bearing lizard occurring along a 1,200-m altitudinal, and climatic, gradient from sea level to highland regions throughout Tasmania[13]. Sex determination is affected by maternal basking opportunity in lowland skinks, analogous to temperature-dependent sex determination in egg-laying reptiles[14]. Thermal conditions representative of a cool year delays birth and result in an overproduction of male offspring whereas thermal conditions representative of warm years result in early birth and a small female bias (Fig. 1a). However, experimental manipulation of female thermal opportunity during gestation (a common garden experiment) reveals that sex determination in highland populations is not affected by temperature (Fig. 1b). This difference in sex-determining systems has consequences for sex ratios at the population level, with a negative correlation between the cohort sex ratio and annual temperature in lowland, but not highland, populations ($r = -0.84$, $P = 0.017$, $N = 7$ and $r = -0.20$, $P = 0.65$, $N = 7$, respectively; slopes differ significantly between populations, $F_{1,10} = 12.8$, $P = 0.005$).

Earlier birth for females may be adaptive because birth date affects opportunity for growth until maturity, which is more important in female than in male snow skinks as a result of differences in selection on body size[14–16]. However, climatic conditions vary substantially across altitudes and the cooler conditions in highland regions induce several changes in lizard life history. High-altitude populations have a shorter activity season, more synchronized birth, slower growth and delayed age at maturity compared to lowland populations[13,17]. Birth date is therefore a relatively unimportant predictor of the onset of maturity and reproductive output at high altitudes (Fig. 2a). Specifically, at low altitudes early-born females have about 50% higher lifetime fitness than late-born females, whereas at high altitudes the effect of birth date on

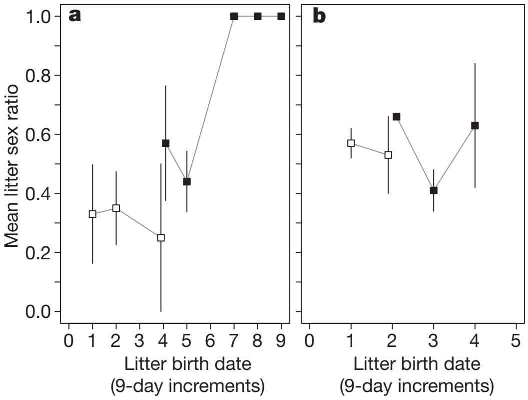

Figure 1 | Experimental effects of thermal conditions on sex ratio and birth date. Sex ratio = male/(male + female). Poor thermal condition during gestation (filled squares) results in delayed birth compared to good thermal condition (open squares), with a corresponding significant effect on offspring sex in lowland (**a**) but not highland (**b**) females. Error bars are s.e.m. Logistic regression with the proportion of males as a dependent variable and treatment and birth date (measured in days from birth) as predictors: birth date for lowland population $\chi^2 = 20.66$, $P = 0.0001$, $N_{\text{females}} = 13, 18$ and for highland population, $\chi^2 = 0.15$, $P = 0.70$, $N_{\text{females}} = 31, 24$.

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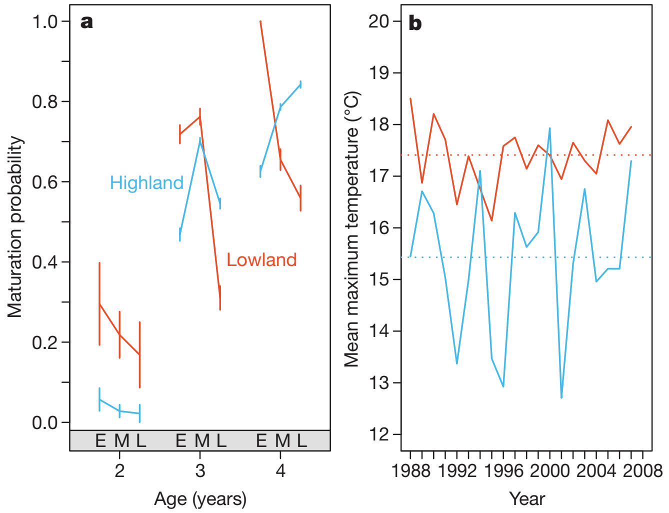

Figure 2 | **Life-history and temperature differences between lowland and highland populations of *N. ocellatus*.** **a**, Probability of maturation (±s.e.m.) at a given age for female offspring in relation to their timing of birth (E, early; M, intermediate; L, late) for lowland (red) and highland (blue) populations. Estimates based on field data from 2000–2007 (details provided in the Supplementary Information). **b**, Annual variation in mean maximum temperature experienced during the first half of gestation for lowland (red) and highland (blue) populations.

female fitness is greatly reduced (Fig. 2a; Supplementary Table 3). Furthermore, highland populations experience relatively high between-year variance in temperature (Fig. 2b), which could select for GSD because it prevents extreme sex ratios and therefore reduces variance in fitness across breeding attempts[1,18–20].

To derive conditions under which the observed evolutionary divergence in sex determination in snow skinks could be favoured by natural selection, and to evaluate the relative importance of climate-induced changes in lizard life history and annual fluctuation in temperature, we constructed an individual-based simulation model based on a sex-determining mechanism recently proposed for lizards[5]. In this model, sex is determined by a threshold polymorphism involving four gene loci (see Supplementary Information for details). Each individual has a genetically determined temperature-dependent rate of regulatory gene expression, which needs to exceed a genetically determined threshold level to trigger male development (Supplementary Fig. 3). This allows evolutionary shifts in sex-determining systems via changes in the regulation of a developmental switch by genetic or environmental input. Both GSD and TSD can therefore be seen as emergent outcomes of selection for canalization of this switch, whereas 'mixed systems'[4,5] occur when canalization is incomplete (Supplementary Information). We parameterized this model with empirical data from long-term studies of two populations at the climatic extremes of the species' distribution and used sensitivity analyses to test whether climatic effects on life histories and the differences in the degree of between-year fluctuation in temperatures between altitudes were sufficient to explain the observed divergence in sex-determining systems. In addition, we calculated how well the temperatures experienced by individual females predicted their sex ratios to assess whether our model accurately captured the correlations observed in natural populations (see Methods and Supplementary Information for further details).

The model generated two primary results, both in close accordance with empirical data (Fig. 3). First, in simulations parameterized with data from the lowland population, sex determination evolved from pure GSD towards a system with a strong temperature effect (Fig. 3b). This

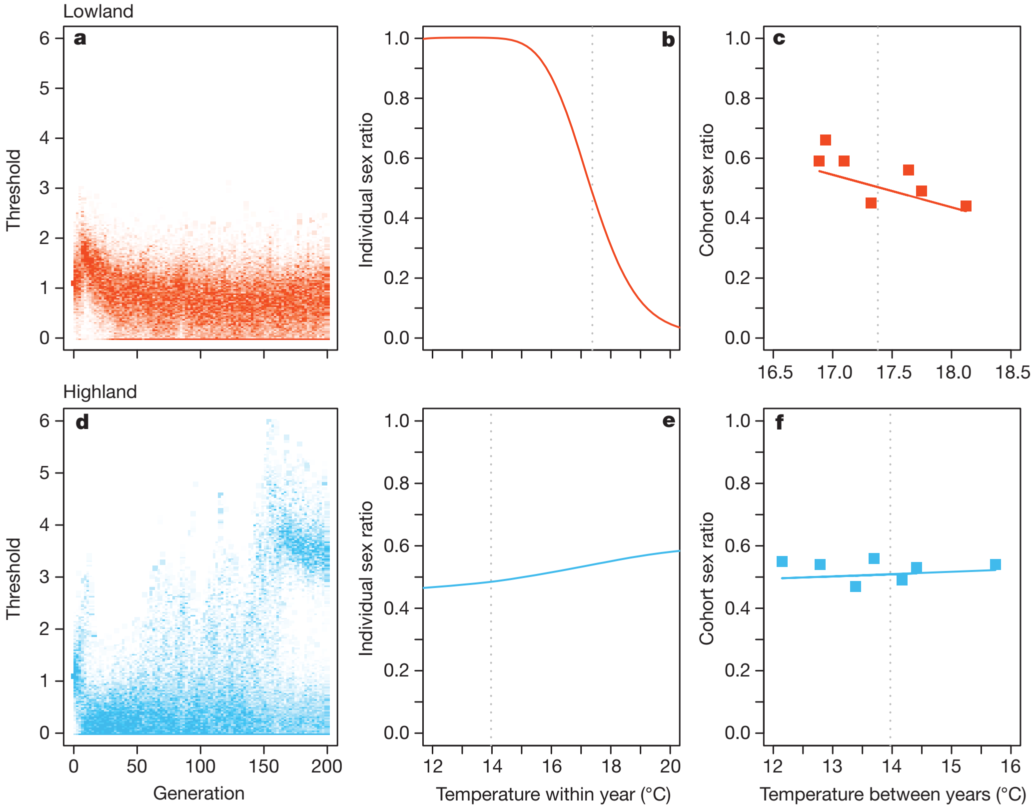

Figure 3 | **Evolutionary simulation results with genetic sex determination as ancestral state.** Upper panels, lowland parameter settings; lower panels, highland parameter settings. **a** and **d**, Population distributions of allelic values at threshold locus changing over time. We note branching in **d** for highland parameter settings, resulting in a novel sex-determining locus: males are 'homozygous' for alleles causing low thresholds and females 'heterozygous' for low and high threshold alleles. **b** and **e**, Evolved average reaction norm for offspring sex ratio as a function of developmental temperature. The vertical dotted line is the average temperature experienced by natural populations. **c** and **f**, Predicted (from evolved reaction norm; line) and observed (natural populations; squares) cohort sex ratios for annual mean maximum temperature in the wild.

generated a significant negative correlation between the cohort sex ratio and average temperature during gestation that closely resembled data from our natural population (Fig. 3c). Second, in simulations parameterized with data from the highland population, sex chromosomes (W or Y) of the initial GSD system were either retained or, if lost, were replaced by a novel genetic element of major effect via disruptive selection on the threshold locus (Fig. 3d). Consequently, the model could generate evolutionary shifts from one sex chromosome system to another—including transitions between male and female heterogamety (Supplementary Information)—but it always produced a sex-determining system that generated average sex ratios that did not deviate substantially from equality, again in close accordance with our natural population (Fig. 3e, f). These results were robust with respect to starting settings, male versus female heterogamety, and linkage between genetic elements (Supplementary Information).

The population divergence in sex-determining systems could be explained by both the increased rate of female maturation with earlier birth date in lowland population and the higher magnitude of annual fluctuations in temperature in the highland population (Supplementary Fig. 4). Thus, a relatively long activity season favours an evolutionary shift from GSD to TSD in lowland populations, manifested in our model through the loss of genes of major effect and adaptive evolution of a sex ratio reaction norm and hence TSD. Conversely, a relatively cold and more variable climate reduces the activity season and delays maturity, which results in minor birth date effects on female age and size at maturity and causes disruptive selection on regulatory elements in sex-determining networks and the emergence of novel sex chromosomes. This model may also capture observed population or species divergence in sex-determining systems in fish[10,12] and thus may be generally applied to short-lived species.

Climate-driven population divergence in sex-determining systems emphasizes a creative role of phenotypic plasticity in evolution[21]. First, the effect of climate on lizard life history is largely a passive result of how thermal opportunity constrains activity patterns rather than an evolved adaptation[22,23]. However, such non-adaptive plasticity can apparently contribute to divergent selection on seasonal sex ratio adjustment and, hence, sex-determining mechanisms across species' distributions. Second, the observation that stressfully high or low temperatures have a causal effect on sex determination also in vertebrates with GSD[5,24] suggests that temperature-induced developmental plasticity can simultaneously expose variation in sex determination and cause novel selection on this variation, thereby greatly facilitating evolutionary divergence in sex-determining systems[21,25]. If so, transitions between sex-determining systems may only require minor secondary modifications in the regulation of gonad differentiation, suggesting substantial scope for interchangeability between genetic and environmental determinants of sex[21].

METHODS SUMMARY

All data are based on field studies of two intensively monitored populations at the climatic extremes of the species' distribution[13,17,26] and from the Bureau of Meteorology station situated close to our study sites. Females undergo gestation in the field and are brought into the laboratory just before birth to enable assessment of sex ratios and reproductive output[26]. The data were used to estimate survival, onset of maturity and reproductive output as a function of birth date to generate parameter estimates for the simulation model (see Supplementary Information). We used the mean daily maximum temperatures during the period of temperature-sensitivity of embryos as our index of thermal opportunity[26,27].

To test directly the effect of thermal opportunity on sex determination we captured females early in gestation from areas adjacent to each of our main study sites and split them into two groups per population: extended basking conditions representative of warm years in lowland populations and limited basking conditions representative of cool years in highland populations (see ref. 14 for further detail).

Our simulation model is polygenic[18] and based on a dosage sex-determining mechanism recently proposed for lizards[5]. Sex is a threshold polymorphism determined by allelic values at four different loci (see Supplementary Information for details). We used daily temperatures from the past 20 years to calculate the long-term yearly mean (T_M) and the annual variation (σ_B) in temperature as well as the within-year variation (σ_W) in temperature. Each of 20 simulations started with 5,000 males and 5,000 females and the same values for reaction norm and threshold loci, with the age set to the minimum age at maturation. All results are from simulations run for 200,000 years.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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1. Bull, J. J. *The Evolution of Sex Determining Systems* (Benjamin/Cummings Inc., 1983).
2. Valenzuela, N. & Lance, V. *Temperature-Dependent Sex Determination in Vertebrates* (Smithsonian Books, 2004).
3. Janzen, F. J. & Phillips, P. C. Exploring the evolution of environmental sex determination, especially in reptiles. *J. Evol. Biol.* **19,** 1775–1784 (2006).
4. Radder, R., Pike, D. A., Quinn, A. E. & Shine, R. Offspring sex in a lizard depends on egg size. *Curr. Biol.* **19,** 1–4 (2009).
5. Quinn, A. E. *et al.* Temperature sex reversal implies sex gene dosage in a reptile. *Science* **316,** 411–416 (2007).
6. Crews, D. & Bull, J. J. Mode and tempo in environmental sex determination in vertebrates. *Semin. Cell Dev. Biol.* **20,** 251–255 (2009).
7. Warner, D. A. & Shine, R. The adaptive significance of temperature-dependent sex determination in a reptile. *Nature* **451,** 566–569 (2008).
8. Uller, T., Pen, I., Wapstra, E., Beukeboom, L. W. & Komdeur, J. The evolution of sex ratios and sex-determining systems. *Trends Ecol. Evol.* **22,** 292–297 (2007).
9. Charnov, E. & Bull, J. J. When is sex environmentally determined? *Nature* **266,** 828–830 (1977).
10. Conover, D. O. Adaptive significance of temperature-dependent sex determination in a fish. *Am. Nat.* **123,** 297–313 (1984).
11. Warner, D. A., Uller, T. & Shine, R. Fitness effects of the timing of hatching may drive the evolution of temperature-dependent sex determination in short-lived lizards. *Evol. Ecol.* **23,** 281–294 (2009).
12. Conover, D. O. & Heins, S. W. Adaptive variation in environmental and genetic sex determination in a fish. *Nature* **326,** 496–498 (1987).
13. Wapstra, E. & Swain, R. Geographic and annual variation in life history traits in a temperate zone Australian skink. *J. Herpetol.* **35,** 194–203 (2001).
14. Wapstra, E. *et al.* Maternal basking behaviour determines offspring sex in a viviparous reptile. *Proc. R. Soc. Lond. B* **271,** S230–S232 (2004).
15. Olsson, M. *et al.* Sexual dimorphism in lizard body shape: the roles of sexual selection and fecundity selection. *Evolution* **56,** 1538–1542 (2002).
16. Atkins, N. *Parental Effects in Two Species of Viviparous Lizards:* Niveoscincus microlepidotus *and* N. ocellatus. PhD thesis, University of Tasmania (2007).
17. Wapstra, E., Swain, R. & O'Reilly, J. M. Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia* **3,** 646–655 (2001).
18. Bulmer, M. G. & Bull, J. J. Models of polygenic sex determination and sex ratio control. *Evolution* **36,** 13–26 (1982).
19. van Dooren, T. J. M. & Leimar, O. The evolution of environmental and genetic sex determination in fluctuating environments. *Evolution* **57,** 2667–2677 (2003).
20. Leimar, O., Hammerstein, P. & van Dooren, T. J. M. A new perspective on developmental plasticity and the principles of adaptive morph determination. *Am. Nat.* **167,** 367–376 (2006).
21. West-Eberhard, M.-J. *Developmental Plasticity and Evolution* (Oxford University Press, 2003).
22. Adolph, S. C. & Porter, W. P. Growth, seasonality, and lizard life histories: age and size at maturity. *Oikos* **77,** 267–278 (1996).
23. Cadby, C. D. *et al.* Multi-scale approach to understanding climate effects on offspring size at birth and date of birth in a reptile. *Integr. Zool.* **5,** 164–175 (2010).
24. Ospina-Alvarez, N. & Pifferer, F. Temperature-dependent sex determination in fish revisited: prevalence, a single sex ratio response pattern, and possible effects of climate change. *PLoS ONE* **3,** E2837 (2008).
25. Uller, T. & Badyaev, A. V. Evolution of "determinants" in sex determination: a novel hypothesis for the origin of environmental contingencies in avian sex-bias. *Semin. Cell Dev. Biol.* **20,** 304–312 (2009).
26. Wapstra, E. *et al.* Climate effects on offspring sex ratio in a viviparous lizard. *J. Anim. Ecol.* **78,** 84–90 (2009).
27. Neaves, L. *et al.* Embryonic gonadal and sexual organ development in a small viviparous skink, *Niveoscincus ocellatus*. *J. Exp. Zool.* **305A,** 74–82 (2006).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Field procedures and data collection. Between 2000/2001 and 2007/2008 approximately 90% of females from one lowland and one highland population of *N. ocellatus* were captured every year at the end of gestation, just before giving birth, resulting in a total of >1,500 females and >4,500 offspring. The taxonomic status of the populations as a single species and details on differences in life history traits have been described elsewhere[13,17,28]. Females were housed in cages until parturition, when all offspring were measured and sexed using hemipene eversion (repeatability >0.98 on the basis of animals followed to sexual maturity)[26]. Sex in this species is determined during the first half of gestation[27]. Offspring were released back into their population of origin randomly at 12 locations within each population. Paternity was assessed in a subset of litters using microsatellites[16]. The field data was used to estimate survival, onset of maturity, and reproductive output as a function of birth date, which were subsequently used as parameter estimates for the simulation model (see below; Supplementary Table 1).

Common garden experiment. Females captured early in gestation (before sex determination is completed[27]) from areas adjacent to each of our main study sites were split into two groups per population: extended basking conditions representative of warm years in lowland populations (10 h of basking per 24 h) and limited basking conditions representative of cool years in highland populations (4 h of basking per 24 h)[13,14]. At parturition, offspring were measured and sexed as for the natural populations. Sex-specific mortality can be ruled out because the number of offspring corresponded to the number of ovulated eggs assessed using palpation.

Climate data. Climatic data was obtained from Bureau of Meteorology stations situated close to our study sites. 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 during gestation (first half of gestation, assigned as 1 October to 15 November in lowland and 15 October to 1 December in highland populations), which is an accurate determinant of the temperature experienced during sex determination[26].

Simulation model. Our model is polygenic[18] and based on a dosage sex-determining system recently proposed for lizards[5]. Sex is a threshold polymorphism determined by allelic values at four different loci (see Supplementary Information for details). On the basis of daily temperatures from the past 20 years (from each altitude) we calculated the long-term yearly mean (T_M) and the annual variation (σ_B) in temperature as well as the within-year variation (σ_W) in temperature. In the model the yearly temperature (T_Y) is calculated at each time step by drawing a value from a normal distribution with mean T_M and standard deviation σ_B. T_Y is further used to calculate female-specific thermal conditions (T_F) by drawing a value from a normal distribution with mean T_Y and standard deviation σ_W. To facilitate model building, we divided each reproductive season into three categories: early, intermediate and late breeding (see Supplementary Information for further detail).

Data from our long-term study of two focal populations were used to estimate the minimum age at maturation, number of offspring, offspring and adult survival, and the probability of breeding at age t (Supplementary Information). Because age and body size do not influence male reproductive success in snow skinks[15,16], we set the effect of birth date on male reproductive fitness to be zero. Each of 20 simulations started with 5,000 males and 5,000 females and the same values for reaction norm and threshold loci, and with the age set to the minimum age at maturation. The life history follows a simple structure (Supplementary Fig. 1). In brief, females mate with a randomly drawn male and produce a number of offspring according to her age drawn from a distribution of clutch sizes. The sex of the offspring is determined by the number of Z (or X) chromosomes, the reaction norm and threshold loci, and T_F (Supplementary Fig. 3). Offspring have a fixed probability of survival to the next year (survival is independent of birth date; Supplementary Information). Offspring that have reached the minimum age at maturation have a fixed age-specific probability of reproducing that depends on their timing of birth. At the end of each time step all individuals in the population age by one year and the cycle is restarted. All results are from simulations run for 200,000 years.

28. Melville, J. & Swain, R. Evolutionary relationships between morphology, performance and habitat openness in the lizard genus *Niveoscincus* (Scincidae: Lyosomaniae). *Biol. J. Linn. Soc.* **70,** 667–680 (2000).