

## LETTER

## Co-occurrence of multiple, supposedly incompatible modes of sex determination in a lizard population

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**Abstract**

Sex is determined genetically in some species (genotypic sex determination, or GSD) and by the environment (environmental sex determination, or ESD) in others. The two systems are generally viewed as incompatible alternatives, but we have found that sex determination in a species of montane lizard (*Bassiana duperreyi*, Scincidae) in south-eastern Australia is simultaneously affected by sex chromosomes and incubation temperatures, as well as being related to egg size. This species has strongly heteromorphic sex chromosomes, and yet incubation at thermal regimes characteristic of cool natural nests generates primarily male offspring. We infer that incubation temperatures can over-ride genetically determined sex in this species, providing a unique opportunity to explore these alternative sex-determining systems within a single population.

**Keywords**

Heteromorphic sex chromosomes, incubation, sex-ratio, temperature-dependent sex determination.

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**INTRODUCTION**

Sex is determined at the time of conception in most kinds of organisms, and depends upon the genetic constitution of the zygote (genotypic sex determination, or GSD). However, a diverse array of taxa delay the time at which an individual's sex is determined until partway through ontogeny. This situation allows sex to be determined in response to some environmental influence (environmental sex determination, or ESD). For example, sex in humans is determined by the X–Y chromosome system, whereas sex in crocodiles is determined by the temperatures that the eggs experience during incubation (Bull 1980). There have been many evolutionary transitions between these two modes of sex determination (Bull 1980). The two systems are often viewed as incompatible alternatives (Janzen & Paukstis 1991; Janzen 1996). However, we have found that sex determination in a species of montane lizard is simultaneously affected by sex chromosomes and incubation temperatures, as well as being related to egg size.

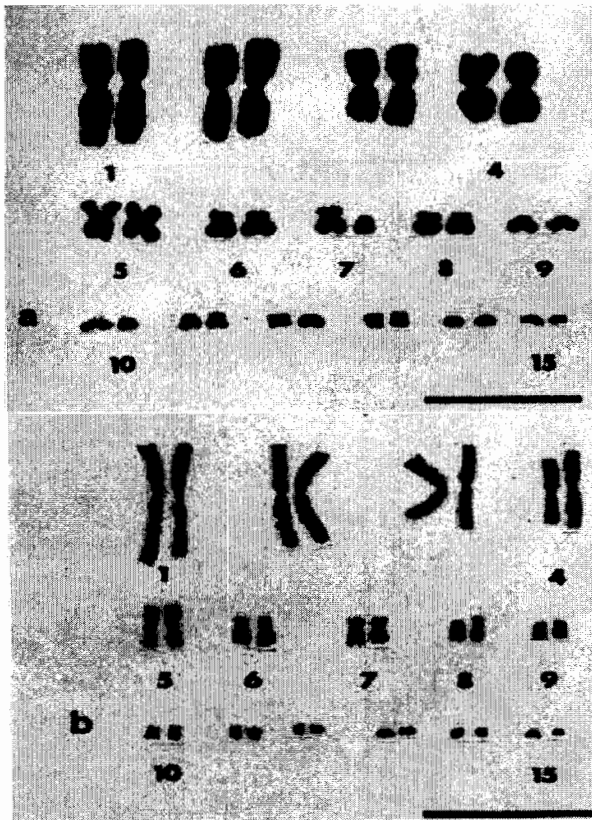
**MATERIALS AND METHODS**

Three-lined skinks (*Bassiana duperreyi*, Scincidae) are medium-sized (to 80 mm snout–vent length) terrestrial

lizards from cool-climate habitats in south-eastern Australia. Females lay clutches of 3–9 eggs under logs and rocks in early summer. This species has heteromorphic sex chromosomes, with males as the heterogametic sex (XY) whereas females are homogametic (XX). Chromosome pair 7 is the heteromorphic pair (Fig. 1), as it is in the two other species of *Bassiana* and in another 28 species from nine other genera of lygosomine skinks (Hardy 1979; Donnellan 1985, 1991; Hutchinson & Donnellan 1992).

As part of a long-term study on phenotypic plasticity in *Bassiana duperreyi*, we have monitored thermal regimes and hatchling phenotypes from >300 natural nests of this species in the Brindabella Range of south-eastern Australia (Shine *et al.* 1997). We have also conducted extensive incubation experiments in the laboratory to examine the ways in which nest temperatures and hydric regimes modify the phenotypic traits of hatchlings (Flatt *et al.* 2001).

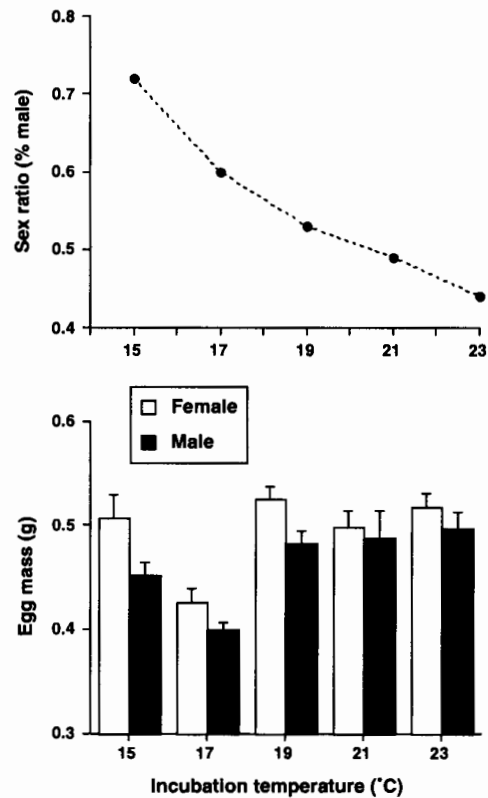
For the present study, we incubated freshly laid eggs collected from natural nests in the field (see Shine *et al.* 1997 for methods) in cycling-temperature incubators in the laboratory. The incubators were set to mimic the diel fluctuations (typically spanning approximately 15 °C) seen in natural nests. The sex of hatchlings was determined by squeezing the tailbase to manually evert hemipenes (Harlow 1996).



**Figure 1** Karyotypes of male and female scincid lizards *Bassiana duperreyi*; note that pair 7 is heteromorphic in (a) the male specimen but not (b) the female.

## RESULTS

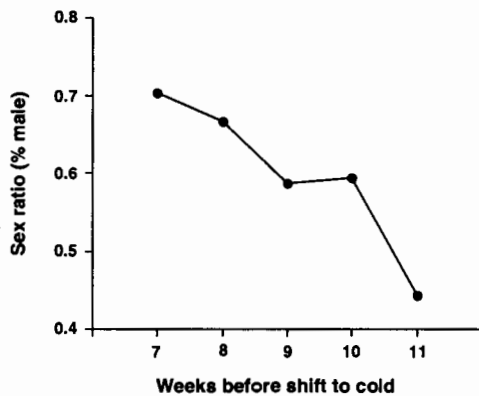
Remarkably, the sex of hatchling *Bassiana duperreyi* is affected by incubation temperature (Fig. 2a) as well as sex chromosomes (Fig. 1). Eggs that were kept at temperatures similar to those experienced in cool summers at high elevations in the field (Shine *et al.* 1997) produced >70% male offspring, whereas thermal regimes characteristic of warmer nests at lower elevations produced sex ratios close to 50 : 50 (Fig. 2a). The sex-ratio skew cannot be attributed to maternal allocation of genetic sons to cool incubation, because the eggs were randomly allocated (by us) to incubation temperatures. Neither can the temperature effect be due to differential mortality of daughters at lower temperatures (Burger & Zappalorti 1988), because the sex ratios at low-temperature incubation were significantly male-biased even if all eggs that failed to hatch (and hence, could not be sexed) were scored as female ( $\chi^2 = 6.48$ , 1 d.f.,  $P < 0.05$ ). Thus, incubation temperatures somehow over-ride chromosomal sex determination at low temperatures. Experiments in



**Figure 2** Sex determination in the scincid lizard *Bassiana duperreyi* is affected by incubation (a) temperature and (b) egg size. (a) Sex ratio of hatchlings from eggs incubated in the laboratory, as a function of mean incubation temperature (based on 726 viable eggs; sample sizes at each temperature = 39, 189, 144, 288, 66 eggs; log-likelihood ratio from logistic regression  $\chi^2 = 10.01$ , 1 d.f.,  $P < 0.0002$ ). (b) Regardless of incubation temperature, the eggs that produced female hatchlings were larger (heavier) than were those that produced males (for 461 female eggs, 536 male eggs;  $F_{1,995} = 7.79$ ,  $P < 0.006$ ). Mean egg sizes varied among treatments because of annual variation in egg sizes and temperatures tested, not because of any biological association between egg size and incubation regime.

which we shifted eggs from warmer to cooler nests indicate that this flexibility in sex determination persists well into incubation, with sex ratios changing even if eggs were shifted >9 weeks into a 12-week incubation period (Fig. 3).

Egg size is also related to sex determination: the eggs that produced daughters were larger than those that produced sons, at all incubation temperatures (Fig. 2b). This might be due to an additional sex-determining mechanism, whereby some cue related to egg size over-rides chromosomal and/or temperature-determined sex. Alternatively, mothers may allocate genetic males to smaller eggs, as occurs in some species of birds (Cordero *et al.* 2000). Regardless, our data



**Figure 3** Effect of shifting eggs to colder conditions (simulating a fall in soil temperatures in autumn) on sex ratios of hatchling scincid lizards (*Bassiana duperryi*). The eggs (124 in number, none of which were included in the data for Fig. 2) were kept at a thermal regime similar to warm nests in the field ( $18 \pm 7.5$  °C diel cycle (Shine *et al.* 1997)) and then progressively shifted to "cool-nest" temperatures (mean of  $15 \pm 7.5$  °C) partway through development. Sex ratios were increasingly male-biased with earlier shifts to low-temperature incubation ( $n = 5$ ,  $r = -0.94$ ,  $P < 0.02$ ).

show that sex in these montane lizards is simultaneously affected by at least two different factors (genes and nest temperatures) and potentially, by egg sizes as well.

## DISCUSSION

What is the adaptive significance (if any) of this complex pattern? Each sex-determining factor plausibly enhances fitness. First, genetic mechanisms will tend to keep the overall sex allocation close to 50 : 50, as favoured by frequency-dependent selection (Fisher 1930). Second, female *Bassiana* benefit more from high-temperature incubation than do their brothers in terms of several phenotypic traits, notably running speeds (Elphick & Shine 1998), and so the observed pattern of temperature sex determination (TSD) matches hatchling sexes to optimal incubation temperatures (Shine *et al.* 1995). Third, female *Bassiana* grow larger than males, and larger females produce more eggs (Pengilley 1972; Greer 1989); thus, larger offspring sizes may benefit daughters more than sons (Trivers & Willard 1973).

Given the frequent phylogenetic transitions that have occurred between GSD and ESD, we expect to see some populations in which sex is determined by a combination of genetic and environmental effects. Indeed, this situation may prove to be widespread. Incubation temperatures have been reported to over-ride genetic sex determination in a few species of salamanders (Dorazi *et al.* 1995) and fishes (Lagomarsino & Conover 1993; Baroiller *et al.* 1995), and

genetic variation in sex-determining temperature thresholds occurs in turtles (Bull *et al.* 1982). However, the system of sex determination in *Bassiana* differs from any that have previously been described. For example, heteromorphic sex chromosomes have not been reported in any other ESD reptile species, and thermal effects on sex determination in this group typically involve steep threshold effects (virtually 100% of one sex or the other at any single incubation temperature (Janzen & Paukstis 1991)), not the gradual shift shown in *Bassiana* (Fig. 2a).

Genetic models suggest that there is no intrinsic difficulty in evolving between genotypic and environmental sex determining mechanisms, except when degenerate sex chromosomes are present (or other forms of sex-specific effects are linked to the sex determining genes: Bull 1983). The problem with degenerate sex chromosomes is that environmental effects may cause the formation of YY individuals. However, because male *Bassiana* are heterogametic, and the environmental effects cause an excess of males, environmental effects will create XX males but not XY females, and hence no YY individuals. There is then no necessary incompatibility between the two mechanisms. Especially in high-elevation sites where incubation temperatures are low, we would expect to find animals that are phenotypically male but genetically female. We are currently engaged in studies to test this prediction.

In summary, our data considerably expand the known diversity of sex-determining systems in reptiles, and show that supposedly incompatible alternatives can indeed coexist. Such cases offer exciting opportunities to clarify the processes that have operated during phylogenetic shifts between sex-determining systems in animals.

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