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Sex differences in optimal incubation temperatures in a scincid lizard species

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Abstract Most theoretical models for the evolution of temperature-dependent sex determination (TSD) rely upon differential fitness of male and female offspring incubated under different thermal regimes. However, there are few convincing data on this topic. We studied incubation effects in a lizard species (*Bassiana duperreyi*, Scincidae) with genotypic sex determination, so that we could separate effects due to incubation temperatures from those due to offspring gender. We incubated eggs under two different fluctuating-temperature regimes that simulated hot and cold natural nest-sites. The effects of our incubation treatments on phenotypes of the hatching lizards (morphology and locomotor performance) differed between the sexes. Females emerging from eggs exposed to the “hot nest” treatment (diel cycling, 23–31°C) were larger, and ran faster, than did their sisters from the “cold nest” treatment (16–24°C). Males showed a smaller and less consistent phenotypic response than females. These incubation-induced responses were relatively stable during the first few weeks of life post-hatching, at least in captive lizards maintained under laboratory conditions. These kinds of sex differences in the phenotypic responses of hatchling reptiles to incubation conditions provide a plausible basis for the evolution of temperature-dependent sex determination in reptiles.

Key words *Bassiana duperreyi* · Embryo · Phenotype · Reptile · Thermal

Introduction

Environmental sex determination (ESD) occurs in both plants and animals, and has stimulated considerable research and speculation from evolutionary biologists

(e.g. Charnov and Bull 1977; Charnov 1982; Rhen and Lang 1995). Although some examples of ESD fit well with theory (e.g. Charnov and Bull 1977; Bull 1980; Bull and Charnov 1988; Bull et al. 1988; Conover and Kynard 1981; Conover 1984), other cases remain an evolutionary enigma, and continue to be the focus of significant controversy (e.g. Bull et al. 1988; Rhen and Lang 1995; Janzen and Paukstis 1991; Janzen 1993, 1996; Roosenburg 1996). Temperature-dependent sex determination (TSD) in reptiles provides such a case. Several alternative explanations for the nature of the adaptive advantage conferred by TSD have been proposed (e.g. Bull 1980; Ewert and Nelson 1991; Burke 1993). These hypotheses invoke a series of assumptions, most of which have little empirical support. In an attempt to clarify the adaptive significance of TSD in reptiles, we set out to evaluate the validity of the most crucial of these assumptions, that optimal incubation temperatures differ between male and female offspring, by means of an experimental study.

Although most research on the evolutionary advantages of TSD has (unsurprisingly) been based on studies of species that exhibit TSD, this approach has the disadvantage of confounding offspring gender with incubation temperature – thus making it difficult to separate effects due to each of these factors (Janzen and Paukstis 1991). Endocrine techniques to override TSD offer one solution to this problem, but may introduce their own artifacts (Rhen and Lang 1995). An alternative approach is to study a species with genotypic sex determination (GSD). This procedure makes it more difficult to extrapolate results to TSD species, but has the advantage of separating gender effects from incubation-temperature effects (e.g. Bull 1980; Webb and Cooper-Preston 1989). We adopted the latter approach, and studied a lizard species that exhibits GSD. We have previously examined phenotypic plasticity in this species (Shine 1995; Shine and Harlow 1996; Elphick and Shine, in press), and provide a fuller description of our study on sex effects (Shine et al. 1995) in the current paper.

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Materials and methods

The montane lizard *Bassiana duperreyi* is a medium-sized (to 80 mm snout-vent length) oviparous diurnal skink that is distributed throughout much of sub-alpine southeastern Australia (Cogger 1992). It has genotypic sex determination (GSD), with heteromorphic sex chromosomes (Donnellan 1985). Extensive laboratory experiments have revealed no effect of incubation temperature on sex ratios of offspring (Shine 1995; Elphick 1995; this study). We chose *B. duperreyi* as our study organism for several reasons. First, because it displays GSD, we can examine the separate (and interactive) effects of gender and incubation temperatures on the phenotype, without needing to artificially decouple these traits via endocrine manipulation of eggs (see Rhen and Lang 1995). We are unaware of any previous attempt to look for sex-by-temperature interactions in any GSD reptile species. Second, because *B. duperreyi* is a fast-growing early-maturing species (maturation in <24 months; Pengilley 1972), it may be easier to identify incubation and sex effects in *B. duperreyi* than in the slow-growing late-maturing species (turtles and crocodylians) that have been the focus of most previous research in this field. Third, because the oviparous *Bassiana* is thought to be the sister-group to the viviparous *Pseudemoia* lineage (Hutchinson et al. 1990), information on the effects of incubation temperature may clarify the hypothesis that phenotypically plastic responses have played a role in this evolutionary transition between alternative reproductive modes (Shine 1995). Fourth, our long-term monitoring has documented substantial thermal variation among natural nests of *B. duperreyi*, through both space and time (Shine and Harlow 1996; Shine et al. 1997), so that we can simulate biologically realistic hot and cold incubation regimes. Fifth, our laboratory work has shown that hatchling phenotypes of *B. duperreyi* are affected by incubation temperatures over this thermal range (e.g. Shine 1995; Shine and Harlow 1996; Shine et al. 1997; Elphick and Shine 1998), so that the system is likely to reveal sex-specific effects if indeed they occur. Lastly, *B. duperreyi* is abundant in suitable habitats, and oviposition is highly synchronous (Pengilley 1972; Shine 1983, 1995; Shine and Harlow 1996).

Over the summer of 1994–1995, we collected recently oviposited eggs from four communal nest sites (total of 83 eggs) at Corcee Flats and Piccadilly Circus (elevation 1050 m and 1246 m respectively) in the Brindabella Ranges, 40 km west of Canberra in the Australian Capital Territory. Thirteen gravid females were also collected at this time. Upon arrival at the University of Sydney, all eggs were weighed (± 0.001 g) on a Sartorius top-loading balance. They were then transferred to individually labelled 64-ml glass jars containing vermiculite (water potential -200 kPa), and sealed with plastic cling wrap to prevent moisture loss throughout incubation. Although water potentials were identical at the beginning of incubation, they would have diverged due to the different temperature treatments (Packard et al. 1993). However, the same phenomenon would be expected to occur in natural nests. All gravid females oviposited <12 days after collection, and their eggs (total = 69) were treated in exactly the same way as the field-laid eggs.

To reduce any bias from nest or maternal effects on hatchling traits, clutches were split randomly between two incubation conditions which mimicked the extremes of thermal fluctuations recorded from natural nests in the field (Shine and Harlow 1996). Two ten-step Clayton programmable incubators were used for this purpose. In one, temperatures ranged from 23 to 31°C in a sinusoidal daily cycle (hot nests) and in the other, from 16 to 24°C (cold nests). Eggs in natural nests that we monitored over the same season mostly hatched at a time intermediate between those we recorded for our hot and cold nests, suggesting that our experimental treatments bracketed the range of thermal regimes in natural nests (Elphick 1995). Egg mortality was low (3 of 69 hot eggs and 6 of 68 cold eggs).

After hatching, we maintained the young lizards individually in plastic containers (22 × 13 × 7 cm) with ad libitum food and water. Each cage contained a thermal gradient from 20°C to >40°C for 10 h per day, to provide an opportunity for behavioural thermo-

regulation. For the rest of the day (when the heating system was switched off), the hatchlings were at room temperature (20 ± 1°C).

We measured (snout-vent length, SVL, and tail length) and weighed the lizards at hatching, and at 2, 4, 6, 14, 20 and 24° weeks of age. They were maintained in captivity throughout this period. Running speeds were measured in a 1-m laboratory raceway. Infrared photo-cells were located at 25-cm intervals along the 4.5-cm-wide raceway, to record the cumulative time taken for lizards to cross each successive infrared beam. This technique enabled us to estimate the lizards' speed over the fastest 25 cm and over 1 m. Each lizard was raced three times, with a rest period of >10 min between successive trials. Lizards that refused to run were lightly touched on the tail with an artist's paintbrush. Running trials were conducted at ages of 2, 6, 14 and 20 weeks, at body temperatures of 15, 20, 25 and 30°C ($\pm 1^\circ\text{C}$) each time. Mean selected body temperatures are close to 30°C for *B. duperreyi*, and lizards are often active in the field with temperatures over the entire range of our test conditions (Shine 1983, as "*Leiopisma trilineatum*"). We determined sex by dissection (presence of ovarian follicles or ellipsoid testes). Our experimental techniques have been described in more detail elsewhere (Elphick 1995; Shine 1995; Shine et al. 1995; Shine and Harlow 1996; Elphick and Shine 1998).

Prior to statistical analysis, we checked all data for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett's test); no significant deviations from these assumptions were evident in natural log-transformed data. All analyses were tested for statistical significance at the $P < 0.05$ level. The problem of non-independence of successive trials on the same animals was addressed by using repeated-measures multivariate analysis of variance (MANOVA) for data on animals up to the age of 6 weeks. We tested morphological variables (mass, SVL, tail length) separately from locomotor speed.

Results

Egg mass

Because field-laid eggs had been laid up to 1 week before collection (based on incubation periods), and had taken up moisture before we collected them, we carried out two analyses of our data on egg masses and incubation periods – one on all eggs, and one restricted to eggs laid in the laboratory (and hence weighed immediately after oviposition). Conclusions were identical from analyses on both data sets. There was no significant difference in the mean mass of eggs producing males or females, no difference in mean egg mass between the hot or cold incubation treatments, and no significant interaction between these factors (Table 1).

Incubation period

Incubation temperatures profoundly affected incubation periods: cold-incubated lizards hatched significantly later than hot-incubated lizards (mean incubation periods of 79.9 versus 32.0 days), but mean incubation periods did not differ between male and female hatchlings, and there was no significant interaction between the effects of incubation temperature and gender (Table 1).

Sex ratios

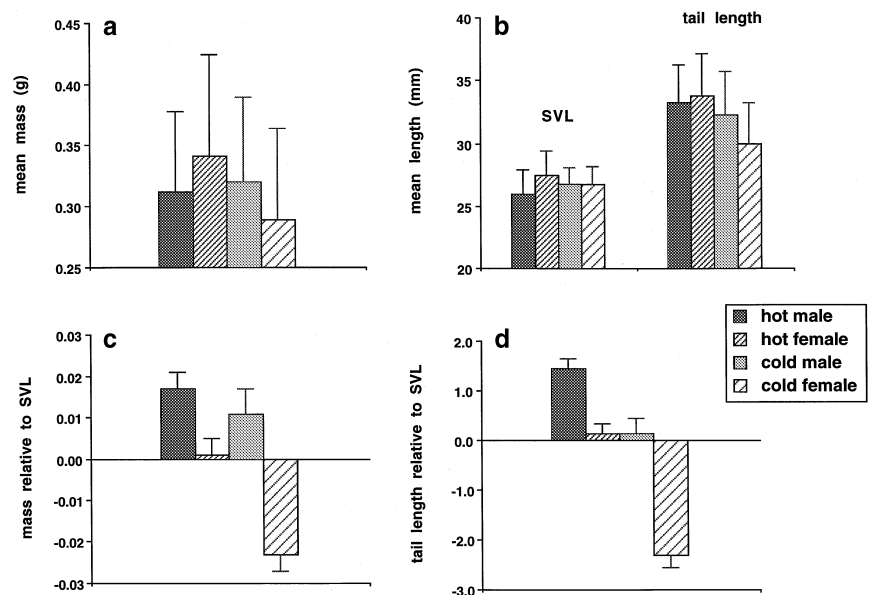
The incubation treatment to which eggs were assigned did not affect hatchling sex ratios (25 females and 20

Table 1 Effects of sex and incubation temperature on phenotypes (size, shape, running speed) of hatchling lizards, *Bassiana duperreyi*. The table provides results from two-factor ANOVAs (first three rows) and repeated-measures MANOVAs on hatchling traits over the first 6 weeks of post-hatching life (all other rows). The MANOVAs incorporated two main effects (sex and incubation temperature), with the interaction between these two traits also of

interest. MANOVAs for locomotor speed also incorporated test temperature as a main effect. The last row shows results from a MANCOVA with hatchling tail length as the covariate, to examine effects of incubation temperature and gender on running speeds relative to hatchling size. Significant ($P < 0.05$) effects are indicated in bold type (SVL snout-vent length)

Hatchling variable	df	Main effect of incubation temperature		Main effect of sex		Interaction between incubation temperature and sex	
		F	P	F	P	F	P
Egg mass (g)	1,35	0.091	0.765	0.531	0.471	0.207	0.652
Incubation period (days)	1,35	1800.39	0.0001	0.479	0.493	1.070	0.308
Survival rates (days to death)	1,79	4.729	0.033	0.003	0.956	0.156	0.694
Hatchling size (SVL, tail length, mass)	3,546	20.339	0.0001	53.544	0.0001	8.635	0.0001
Hatchling speed (over 1 m and over 25 cm)	2,448	166.800	0.0001	0.653	0.522	11.666	0.0001
Hatchling speed relative to tail length	2,432	22.14	0.0001	19.955	0.0001	4.08	0.018

Fig. 1a–d Effects of sex and incubation treatment (“hot” and “cold” thermal regimes) on the size and shape of hatchling lizards, *Bassiana duperreyi*. These graphs show mean values from two-factor repeated-measures ANOVAs for trait values over the first 6 weeks of life. The dependent variables for the two lower graphs are residual scores from general linear regressions of mass and tail length relative to snout-vent length (SVL). Error bars show standard errors



males from the hot treatment, versus 21 females and 20 males from cold; $\chi^2 = 0.16$, $df = 1$, $P = 0.85$).

Offspring size

Male and female offspring were similar in mean sizes (mass, SVL or tail length) overall, but cold-incubated males were larger than cold-incubated females for all three of these traits, whereas the reverse was true for

hatchlings from the hot treatment group (Fig. 1, Table 1). Consequently, the interaction between gender and incubation temperature was significant for all three size descriptors (Table 1).

Morphometric ratios

Both the incubation history and gender of *B. duperreyi* offspring affected relative bodily proportions (mass and

tail length relative to SVL), but without any significant interactions between these effects (Fig. 1, Table 1).

Offspring mortality

There were no significant differences between male versus female survivorship over the 24-week post-hatching period within either of the incubation treatments (Kaplan-Meier method with Mantel-Cox logrank test: hot females versus hot males, $\chi^2 = 0.77$, $df = 1$, $P = 0.38$; cold females versus cold males, $\chi^2 = 0.03$, $df = 1$, $P = 0.85$). However, lizards incubated in the hot treatment survived significantly longer than their cold-incubated siblings (means of 112 versus 88 days). Sex did not affect survival rates, nor was there any significant interaction between these two factors (Table 1).

Growth rates

Growth rates of the young lizards varied as a function of incubation treatment (hot-incubated hatchlings grew faster) and sex (females grew faster), but with no significant interaction between these two factors (Table 1; Fig. 2).

Running speeds

Over both distances tested (1 m and 0.25 m), mean running speeds were similar in males and females, but were significantly affected by test temperature and by the lizard's incubation treatment, as well as by an interaction between incubation treatment and gender. Lizards

from the hot incubation treatment always ran faster than same-sex lizards from the cold treatment group (Table 1). At all test temperatures and over both distances used for our analyses, hot-incubated females always ran faster than hot-incubated males whereas cold-incubated males always ran faster than cold-incubated females (Fig. 2, Table 1).

These locomotor differences were not a secondary consequence of morphological effects; the interaction terms between sex and temperature remained significant even when the data were analysed by three-factor MANCOVA [with sex, incubation temperature and test temperature as factors, hatchling size (tail length) as the covariate, and speed as the dependent variable: Table 1].

Relationship between egg mass and other variables

Our analyses suggest that the gender of a young lizard does not affect the way in which its initial egg mass translates into its body size and running speeds at later ages. Our analyses showed that hatchling body size (mass, snout-vent length and tail length, all relative to egg mass) was affected by incubation treatment and by gender, but with no significant interaction between incubation treatment and sex (Table 2). Gender did not affect running speeds relative to egg mass (Table 2).

Degree of phenotypic plasticity in male and female offspring

The phenotypes of female hatchlings were more strongly affected by incubation temperatures (i.e. showed greater phenotypic plasticity) than were those of their brothers,

Fig. 2 Effects of sex and incubation treatment (hot and cold thermal regimes) on **a, b** the growth rates and **c, d** running speeds of hatchling lizards, *B. duperreyi*. These graphs show mean values from two-factor repeated-measures ANOVAs for trait values over the first 6 weeks of life. **c, d** Data on running speeds over two distances (0.25 and 1.0 m) at four different test temperatures. Error bars show standard errors

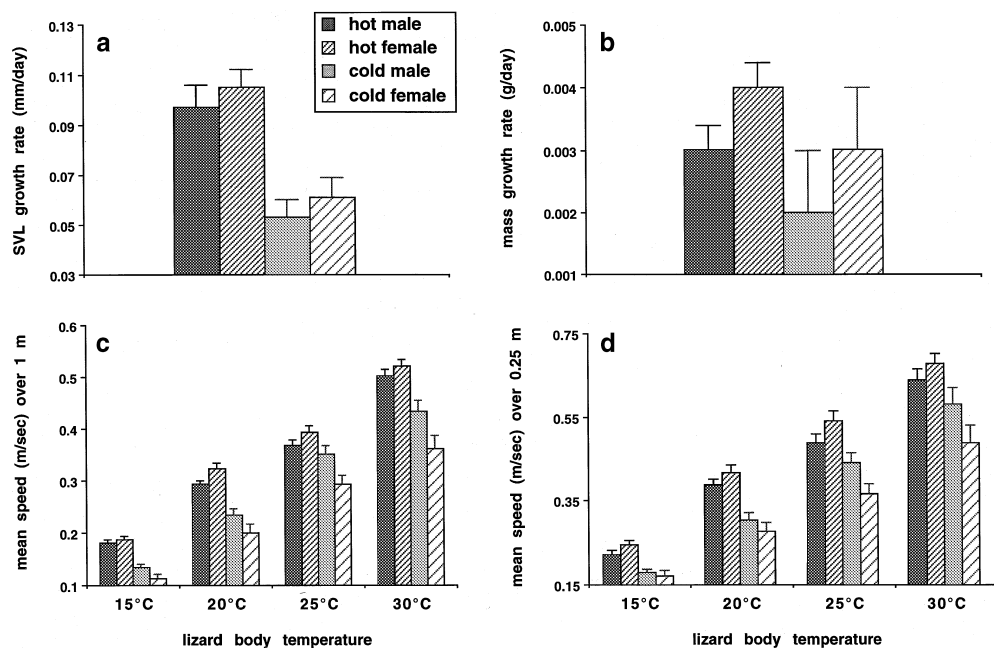


Table 2 Effects of sex and incubation temperature on the relationship between egg mass and the phenotypes (body sizes and running speeds) of hatchling lizards, *B. duperreyi*. The table provides results from repeated-measures MANCOVAs on hatchling traits over the first 6 weeks of post-hatching life, correcting for

initial egg mass. The MANCOVAs incorporated two main effects (sex and incubation temperature; plus test temperature for the running speed MANOVA), with the interaction between the first two traits also of interest

Hatchling variable	df	Main effect of incubation temperature		Main effect of sex		Interaction between incubation temperature and sex	
		F	P	F	P	F	P
Hatchling size (mass, SVL, tail length)	3,542	4.726	0.003	4.332	0.005	0.481	0.696
Hatchling running speed (over 1 m and 0.25 m)	2,432	16.96	0.0001	0.286	0.751	0.232	0.793

particularly for locomotor performance. A paired *t*-test on the proportional change in mean locomotor speeds between same-sex sibling hot and cold-incubated lizards showed much higher values for females than for males ($t = 4.83$, 29 *df*, $P < 0.0001$). However, the same comparison was not quite significant for morphological variables ($t = 2.03$, 24 *df*, $P = 0.054$). Females also showed a greater consistency in the direction of the phenotypic difference induced by incubation temperatures. Females from high-temperature incubation were larger than their cold-incubated sisters in 32 of 42 comparisons (81%), versus 27 of 46 comparisons in males (59%; $\chi^2 = 2.30$, 1 *df*, $P = 0.13$). Similarly, hot-incubated females were consistently faster than cold-incubated females (32 of 32 comparisons, = 100%) whereas hot-incubated males showed a less consistent superiority in speed (25 of 32, = 78%; $\chi^2 = 7.19$, 1 *df*, $P < 0.008$).

Discussion

The clear result from our analyses is that the phenotypes of male and female *B. duperreyi* respond differently to the thermal regimes that the lizards experience during incubation. The phenotypes of female hatchlings are more sensitive in this respect (i.e. more plastic; less canalized) than are the phenotypes of their brothers. Compared to low-temperature incubation, development at relatively high temperatures (i.e. those typical of hot nests in the field) substantially increased the body sizes, growth rates and locomotor performance of female hatchlings. Males showed these effects less consistently and to a lesser degree. The sexes also differed in the way in which egg size affected offspring morphology, but not in the way in which the hatchling's incubation history influenced the relationship between egg mass and hatchling phenotype.

In total, these results document a strong interaction between two factors – incubation temperature and gender – in determining the phenotypic traits of hatchling reptiles. Many workers have shown that incubation re-

gimes influence the phenotypes of hatchling reptiles (see e.g. Webb and Cooper-Preston 1989; Rhen and Lang 1995), including *B. duperreyi* (Shine 1995; Shine and Harlow 1996; Shine et al. 1995; Elphick 1995). Sex differences in the phenotypic traits of hatchlings are also widespread in reptiles (e.g. Kopstein 1941; Clark 1963; Shine 1993). However, *B. duperreyi* is the first species in which incubation temperature and sex have been shown to interact in determining the phenotypes of hatchlings. Our recent studies on natural nests indicate that the same interaction affects hatchling *B. duperreyi* in the wild (Shine et al. 1997).

The assumption that the phenotype at hatching is affected by incubation temperature differently in males and females is a crucial component of some of the most widely-cited theoretical models for the evolution of reptilian TSD (e.g. Burger and Zappalorti 1988; Tousignant and Crews 1995). Our data on *B. duperreyi* provide the most extensive support to date for this assumption, but the same phenomenon has been shown in other reptiles as well. For example, Burger and Zappalorti (1988) demonstrated sex-specific mortality of snake eggs at different incubation temperatures. Other studies show that growth rates of alligators and geckos are affected by both sex and incubation temperature (Joanen et al. 1987; Tousignant et al. 1995). Another report claimed to show “significant selection on the interaction of gender and incubation temperature” on survival rates of hatchling turtles (Janzen 1995, p. 868). However, inspection of Janzen's analysis reveals that he simply demonstrates heterogeneity in survival rates of hatchlings belonging to different sex \times incubation temperature categories. Because Janzen's experimental design confounds sex and temperature, his data are entirely consistent with the hypothesis that hatchling survival depends on incubation temperature but not sex, and with no significant interaction between sex and incubation temperature.

The scarcity of data on sex \times incubation effects on hatchling phenotypes makes it difficult to draw any generalizations about the nature of these effects. The major difference between our male and female *B. duperreyi* hatchlings lay in the degree of their plasticity in

response to incubation conditions. Females showed more plastic responses than males, and hotter incubation appeared to produce “better” female hatchlings. In contrast, female mortality rates were higher at higher incubation temperatures in the pine snake *Pituophis melanoleucus* (Burger and Zappalorti 1988). In the few TSD lizard species studied to date, males are produced at intermediate temperatures, and females at the cooler and hotter extremes (Viets et al. 1993; Harlow 1994; Tousignant et al. 1995; contra Janzen 1996). These data suggest a diversity in the ways in which particular incubation temperatures influence hatchlings of each sex, and further data will be necessary before any conclusions can be drawn. This kind of detail may well have biological implications; for example, it means that the sexes may differ in their sensitivity to “bad” nest conditions. Similar sex differences in responsiveness to hormonal factors in utero have recently been demonstrated in other lizards (Sinervo and Doughty 1996).

Obviously, some caveats are in order. We have not demonstrated any adaptive significance for TSD in reptiles, but have merely clarified the plausibility of models that seek to explain TSD in adaptive terms. Because we have used a GSD study species, it may be that the phenotypic responses to incubation temperatures we have seen are quite different to those exhibited by TSD species. Indeed, one could interpret our data as counter to, rather than supportive of, current explanations for the evolution of TSD. If such responses provide an adaptive basis for TSD, then one could argue that they would be expected to occur only in TSD species. Any GSD species that displays such responses (such as *B. duperreyi*) then offers a contradiction to theory, because we would have expected TSD to evolve in this taxon. This argument is relatively weak, however, because the occurrence of heteromorphic sex chromosomes in *B. duperreyi* (Donnellan 1985) presumably precludes such an evolutionary switch in sex-determining systems (Bull 1980).

Incubation effects seem to be widespread in TSD as well as GSD species (Rhen and Lang 1995), but we do not yet know if sex \times incubation regime interactions occur in TSD species also. We suspect that incubation conditions influence later phenotypes in complex ways in many (perhaps all) reptiles, and that the evolution of TSD involves the exploitation of existing effects in ways that maximize maternal fitness. Although most studies have used artificial incubation regimes (e.g. constant-temperature), this restriction does not necessarily invalidate the general conclusion that thermal regimes influence hatchling phenotypes. An increasing number of studies have shown that the same kinds of effects arise after incubation under the thermal conditions experienced in natural nests (e.g. Shine et al. 1997). A more important caveat is that the experimental studies to date have detected effects on phenotypes, but not on lifetime reproductive success (let alone the fitness of alternative sex-determining systems). Again, the link to fitness is plausible but undocumented.

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