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Research article

Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination

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Abstract. Theoretical models suggest that in changing environments natural selection on two traits, maternal nesting behaviour and pivotal temperatures (those that divide the sexes) is important for maintaining viable offspring sex ratios in species with environmental sex determination (ESD). Empirical evidence, however, is lacking. In this paper, we provide such evidence from a study of clinal variation in four sex-determining traits (maternal nesting behaviour, pivotal temperatures, nesting phenology, and nest depth) in *Physignathus lesueurii*, a wide-ranging ESD lizard inhabiting eastern Australia. Despite marked differences in air and soil temperatures across our five study sites spanning 19° latitude and 1200 m in elevation, nest temperatures did not differ significantly among sites. Lizards compensated for climatic differences chiefly by selecting more open nest sites with higher incident radiation at cooler sites. Clinal variation in the onset of nesting also compensated for climatic differences, but to a lesser extent. There was no evidence of compensation through pivotal temperatures or nest depth. More broadly, our results extend to the egg stage the life history prediction that behaviour is the chief compensatory mechanism for climatic differences experienced by species spanning environmental extremes. Furthermore, our study was unique in revealing that nest site choice influenced mainly the daily range in nest temperatures, rather than mean temperatures, in a shallow-nesting reptile. Finally, indirect evidence suggests that the cue used by nesting lizards was radiation or temperature (through basking or assessing substrate temperatures), not visual detection of canopy openness. We conclude that maternal nesting behaviour and nesting phenology are traits subject to sex ratio selection in *P. lesueurii*, and thus, must be considered among the repertoire of ESD species for responding to climate change.

Key words: lizard, nest depth, nest site choice, nesting phenology, *Physignathus*, pivotal temperature, temperature-dependent sex determination, water dragon

Introduction

Sex determination in vertebrates is traditionally seen as being under strict genetic control, with the sex of an individual determined as the outcome of Mendelian segregation of sex chromosomes (Ohno, 1967). Offspring sex ratio

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in such systems is under strong constraint (Williams, 1979), and closely aligned with the 1:1 sex ratio that is the stable equilibrium condition under most models of sex ratio evolution (Fisher, 1930; Shaw and Mohler, 1953; Charnov, 1982). Relatively recent work on reptiles and fish has shown many to have environmental sex determination, whereby offspring sex is determined by environmental conditions that prevail during development (reviewed by Bull, 1980; Ewert *et al.*, 1994; Conover, 2004). In reptiles, the environmental determinant is temperature, and so we speak of temperature dependent sex determination (TSD). It is a threshold system (Roff, 1996), where one sex is produced predominantly under one set of temperatures and the other sex is produced under the remaining temperatures, with one or two very narrow ranges of temperature, the pivotal temperatures, producing both sexes in relatively equal proportions (Ewert *et al.*, 1994).

The sex ratio of reptiles with TSD can vary dramatically from clutch to clutch in natural nests. Warmer exposed nests or those laid in the warmer part of the nesting season produce dramatically different sex ratios from those in more shaded locations or laid in the cooler parts of the season (Janzen, 1994a; Roosenburg, 1996; Janzen and Morjan, 2001; Doody *et al.*, 2004; St Juliana *et al.*, 2004). Indeed, a high proportion of nests of TSD reptiles produce only one sex (reviewed by Ewert *et al.*, 1994). Variation in climate, either spatially over latitudes or altitudes, or temporally as the climate changes, can be expected to impact offspring sex ratios. Were it not for the self-correcting nature of the sex ratio, a focus of attention since the time of Darwin (1871) and stimulated by the more recent work of Fisher (1930), species with TSD would be vulnerable to extinction as sex ratios moved beyond the limits of demographic viability (Girondot *et al.*, 2004).

Reptiles with TSD can respond, under frequency dependent selection, to bias in the production of the sexes by a shift in the maternal traits of nest site selection (nesting in warmer or cooler localities, Janzen, 1994a; Roosenburg, 1996; Janzen and Morjan, 2001), phenology (nesting earlier or later in the season, Doody *et al.*, 2004) or nest site construction (nesting deeper or shallower to influence exposure to diel variation, Georges, 1989, 1992). The response may be slow through selection for fixed attributes, or rapid through the evolution of facultative capacity.

Demonstrating the action of frequency dependent selection on these traits is difficult, and has only rarely been achieved (Conover and Van Voorhees, 1990; Carvalho *et al.*, 1998). Instead, we commonly rely upon testing predictions by observing patterns in nature. Species with TSD whose ranges span considerable latitudes or elevations provide such an opportunity. In the context of spatial climatic variation, we might predict adjustment in maternal traits influencing where, when and how TSD reptiles deposit their eggs in nests to compensate for increasing or decreasing temperatures, or adjustment in the zygotic trait, pivotal temperature, in order to balance sex ratios across the range. Adjustment in pivotal temperature with latitude has been studied extensively (reviewed in Ewert *et al.*, 2004), and shown to be resistant to change (Bull *et al.*, 1982a; Janzen, 1992; Mrosovsky *et al.*, 2002), or where there is a trend, it is in the opposite direction to that predicted by theory (Bull *et al.*, 1982b; Ewert *et al.*, 1994, 2005). Comprehensive *studies* of latitudinal variation in other potential sex-determining traits such as maternal nesting behaviour, nesting phenology, or nest depth, are lacking (but see Ewert *et al.*, 2005).

The Australian water dragon (*Physignathus lesueurii*) is an ideal candidate for studies of how TSD species compensate for spatial climate variation. The species ranges from hot tropical to cool temperate latitudes and from sea level to 1250 m in elevation (Cogger, 2000; Harlow, 2001). It is abundant, its nests are localised are readily located, and clutches are large enough to allow them to be split across treatments. The foundation laboratory research on its sex determination has been undertaken (Harlow, 2001).

In this paper, we test the hypotheses that clinal variation in four traits is in the appropriate direction and of sufficient magnitude to compensate for climatic differences between latitudes and altitudes. We quantify clinal variation in maternal nesting behaviour and pivotal temperature, the two traits theorised to be important in sex ratio evolution in TSD animals (Morjan, 2003), and two neglected traits: nest depth and nesting phenology. Specifically, we combine pivotal temperature experiments with hemispherical photography and gap light analysis (Fraser *et al.*, 1999), a novel application for quantifying nest site choice (Shine *et al.*, 2003), to investigate clinal variation in nest site choice. We also determine the relationship of nest site choice to nest temperature, our surrogate for offspring sex.

Materials and methods

Study sites and species

The water dragon, *Physignathus lesueurii*, is a large agamid lizard associated with aquatic habitats in eastern Australia. The species is common and occurs from the wet tropics of northern Queensland to cold temperate areas of Victoria (Cogger, 2000). Water dragons possess pattern II TSD (Ewert *et al.*, 1994), with females produced at hot and cool temperatures, and males produced at intermediate temperatures (Harlow, 2001). Nesting occurs in spring and summer in open areas relatively free of shading vegetation, often in sandy substrates (Harlow, 2001). Nesting has not been well-quantified, but water dragons lay their eggs in an excavated chamber (Harlow and Harlow, 1997; Harlow, 2001). In Sydney (roughly mid-way through the N–S geographic distribution of *P. lesueurii*) two clutches of about 4–12 eggs are laid twice in a year, although a few individuals may nest three times (Hay, 1972; Harlow and Harlow, 1997; Harlow, 2001). Also in Sydney, daily variation in nest temperatures spans about 12° C, and hatchlings emerge in summer or autumn after incubation times of about 60–120 days, depending on incubation temperature (Harlow, 2001).

We surveyed for clinal variation in sex-determining traits along a latitudinal gradient spanning 19° latitude and ca. 1200 m in elevation in eastern Australia during September 2003–March 2004 (Fig. 1). The five sites were, from north to south, Cairns, Brisbane, Sydney, Canberra and southern highlands. The three southernmost sites also reflect an elevational gradient: Sydney is near sea level (38 m) while Canberra is near 600 m and the southern highlands range from 950 to 1250 m. Our sites were chosen to cover a wide range of air and soil temperatures during the time when developing eggs are in the ground (Fig. 1). For example, the mean range in monthly air temperature (Sep–Apr) at the

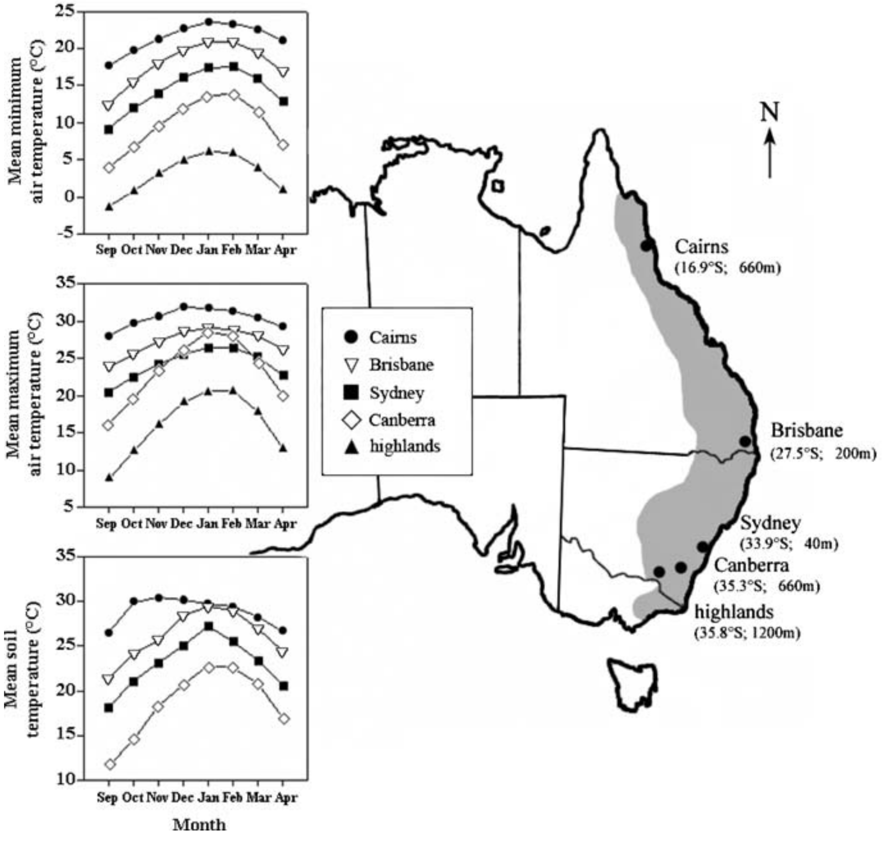

Figure 1. Location of study sites and the distribution of *Physignathus lesueurii*, and mean monthly air and soil temperatures for the sites (data from Australian Bureau of Meteorology). Soil data are from 10 cm depth, and were not available for the highlands sites.

coldest site was 3.1–16.1 °C, compared to 21.5–30.4 °C at the warmest site (Bureau of Meteorology, unpubl. data). We studied 16 populations in total, including 2–7 populations at each site. Populations inhabited a diversity of habitats including rainforest, wet and dry sclerophyl forest, and botanical gardens, but in all cases sites were associated with water (river, stream or pond). The botanic gardens sites were similar to surrounding natural areas in being dominated by native trees but different in possessing man-made watercourses (lagoons and pools) and beds with some non-native understory. Populations within sites were chosen arbitrarily based on our ability to find large numbers of nests.

Nest location, nest site characteristics and nest temperatures

Nests were located by searching open areas along watercourses, noting trial digs by lizards, and searching the immediate area using a homemade probe. The probe facilitated detection of the soft sand back-filled by nesting lizards, and allowed us to find large numbers of nests ($>$ 300) in a short period of time. Although water dragons are known to nest only in open areas (Harlow, 2001), we searched more shaded areas to be sure. Lay date was determined by surveying each site in consecutive days, and a pilot study determined the approximate timing of nesting for each site. To accurately determine when nesting began, one of us was present at each site prior to the onset of nesting. Nests were carefully excavated to measure depth to the top egg, and in fresh nests we employed temperature data loggers (Hobotemp, Onset Comp. Corp.) into the core of the nest. Nests were considered to be fresh if they were $<$ 5 days old, and this was determined using consecutive surveys. Five-days was used because preliminary work revealed that eggs of this age have not yet entered the thermosensitive period (P. Harlow, unpubl. data; S. Doody, unpubl. data). Data loggers recorded temperature at hourly intervals throughout incubation.

Canopy openness and incident radiation of nest sites

To assess the choices made by nesting lizards we compared canopy openness and incident radiation intensity between nest sites and 10–50 randomly selected points (depending on the number of habitats at each site). Canopy openness and incident radiation intensity were estimated using hemispherical photography and a computer program called Gap Light Analyser. We used a Nikon 995 Camera and a Nikon FC-E8 Fisheye lens to take hemispherical photographs. All photographs were taken during the last hour before sunset because reflected sunlight from leaves, trees, and hills introduces error in the estimate of openness (and therefore of radiation). The camera was placed on the ground at each nest site, levelled by eye, and oriented toward due north with a compass. Consistency in orientation is important in the subsequent analysis of the sun's path through the canopy. Hemispherical photographs were then analysed using Gap Light Analyser Version 2.0 (Frazer *et al.*, 1999). This program estimates canopy openness and incident radiation intensity from each photograph (nest site). Incident radiation intensity is calculated from extraterrestrial radiation and the path of the sun relative to canopy gaps, and is facilitated by data on latitude, elevation, and season, inputted by the user. For season we used the lay date plus 90 days to approximate the developmental period of *P. lesueurii*, because we were interested in radiation received by the nest over that period (output is in $MJ/m^2/day$). We also calculated the cloudiness index for each site because we were concerned that, for example, our rainforest sites at Cairns differed in cloudiness (and thus incident radiation at the nest) from other sites. Cloudiness index was calculated using the mean monthly number of sunshine hours for each site (from Australian Bureau of Meterology, unpubl. data).

Pivotal temperature experiments

Eggs from all sites were included except for the Cairns site, owing to insufficient numbers of eggs. Only eggs laid within 5 days of the allocation date were used to ensure that embryos had not entered the thermosensitive period (TSP). The TSP is not precisely known for *P. lesueurii*, but in the lizard *Lacerta vivipara* the outline of the penes begins to form at stage 31 and by stage 36 are gender specific (Dufaure and Hubert, 1961). The earliest known stage of embryonic development when sex is determined is stage 32 (Dufaure and Hubert, 1961; Bull, 1987). To confirm that experimental embryos had not entered the TSP at the time of allocation one embryo from each clutch was removed from the egg, stained with alcoholic Eosin, staged in the laboratory using a dissecting scope following the criteria of Dufaure and Hubert (1961), and stored in 70% ethanol. Clutches containing embryos beyond stage 31 were excluded from the analysis.

Eggs were allocated across six temperature treatments: constant temperatures of 25–30 °C at one-degree intervals in Thermoline® incubators. These temperatures were known to span both pivotal temperatures in the species (Harlow, 2001; Doody, unpubl. data). Temperatures were monitored daily using thermometers calibrated against a reference thermometer certified as accurate by the National Association of Testing Agencies. Eggs were incubated in a substrate of moistened vermiculite (five parts water to four parts vermiculite by mass), and housed in plastic boxes that were enclosed within ziploc plastic bags to keep moisture levels constant. Boxes were monitored for hatchlings daily near the end of embryonic development (minimum∼50 days, Harlow, 2001).

Hatchlings were sexed by everting the hemipenes in males (Harlow, 1996). We determined the sex of each lizard twice without knowledge of temperature treatment (blind). In a few cases we could not definitively assign a sex because hemipenes were diminutive or female-like (females have two small spurs where males have hemipenes, Harlow, 1996). In these cases hatchlings were killed by intra-cranial injection with Nembutal®, and sex was determined using a dissecting microscope by noting the shape and structure of the gonads and presence/absence of Mullerian ducts.

Analysis

Statistical analyses were performed using SAS (SAS Institute, 1998) or Systat 8.0 (SYSTAT, 1998). A two-factor ANOVA was used to compare the means of mean monthly minimum air temperature, mean monthly maximum air temperatures, and mean monthly soil temperatures among sites. In this analysis we only reported the factor of interest = SITE (MONTH was a 'nuisance' factor). Because the monthly data were repeated for each site, we needed to take out variation among months common to each site before undertaking a test of the factor SITE. All other ANOVA's were one-factor. Regression analysis was used to analyse relationships among canopy openness, incident radiation intensity, nest temperatures, air temperatures (minimum and maximum monthly), and soil temperatures. Data on sex ratios in relation to temperature were analysed with logistic regression, including comparisons among sites.

Results

Climate differences and nest temperatures among sites

Air temperatures (Bureau of Meteorology, long-term unpubl. data) were consistently and significantly cooler at higher than at lower latitude sites during each month when *P. lesueurii* eggs were incubating (Fig. 1; mean monthly maximum air temperatures: $F_{4,12} = 103.70$, $p < 0.0001$; mean monthly minimum air temperatures: $F_{4,12} = 946.28$, $p < 0.0001$). Mean monthly soil temperatures (at 10 cm depth, Bureau of Meteorology, long-term unpubl. data) exhibited the same trend (Fig. 1; $F_{3,11} = 72.96$, $p < 0.0001$), although no data were available from the highlands sites.

We obtained nest temperature data from 35 nests, but Cairns was excluded from statistical analyses because of low sample size due to a systematic data logger failure (Table 1). Despite the substantial differences in climate, mean nest temperatures, pooled for each site due to unequal sample sizes, were not related to mean monthly maximum air temperature (Fig. 2; $r^2 = 0.38$,

Table 1. Descriptors of nest temperatures from 36 *P. lesueurii* nests

Site	Mean temperature	Mean daily range	N
Brisbane	26.0 ± 1.00	5.8 ± 1.59	5
Sydney	25.2 ± 0.62	6.3 ± 0.74	7
Canberra	28.0 ± 1.08	9.4 ± 1.73	13
Highlands	23.7 ± 1.08	10.2 ± 1.55	9

Data are means ± 1 SD.

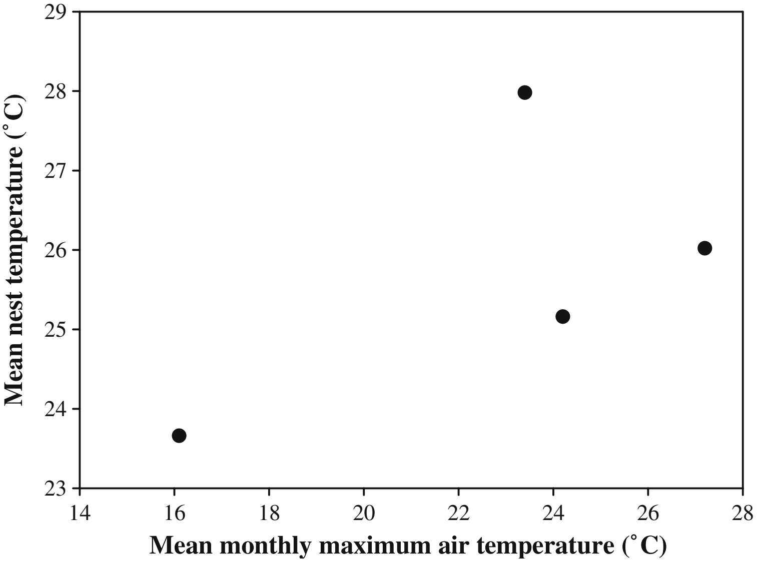

Figure 2. Mean nest temperatures were not significantly related to air temperatures at the sites. Nest temperature data were continuous nest temperatures taken by data loggers at one-hour intervals throughout incubation.

$F_{1,3} = 1.22$, $p = 0.384$), to mean monthly minimum air temperature ($r^2 = 0.16$, $F_{1,3} = 0.37$, $p = 0.604$), or to mean monthly soil temperature at 10 cm depth at each site ($r^2 = 0.56$, $F_{1,2} = 1.26$, $p = 0.464$). This indicates that lizards compensated for climate differences in their choice of a nest site. Although trends were evident (Table 1), mean daily range in nest temperatures was not significantly related to mean monthly maximum air temperature ($r^2 = 0.70$, $F_{1,3} = 4.71$, $p = 0.162$), to mean monthly minimum air temperature ($r^2 = 0.90$, $F_{1,3} = 17.46$, $p = 0.053$), or to mean monthly soil temperature at 10 cm depth at each site ($r^2 = 0.91$, $F_{1,2} = 10.27$, $p = 0.193$).

Canopy openness and incident radiation intensity of nest sites

We determined canopy openness and incident radiation intensity for 305 *P. lesueurii* nests across the five sites (Cairns = 46; Brisbane = 111; Sydney = 67;

Canberra = 51 and highlands = 30). Canopy openness at nest sites, was significantly inversely related to mean monthly maximum air temperature (Fig. 3a; $r^2 = 0.48$, $F_{1,16} = 13.72$, $p = 0.002$), mean monthly minimum air temperature ($r^2 = 0.58$, $F_{1,16} = 18.90$, $p < 0.0001$), and mean monthly soil temperature at 10 cm depth ($r^2 = 0.61$, $F_{1,14} = 18.52$, $p = 0.001$). Openness of nest sites differed from random at some sites but not others (Table 2). This mixture of results seems to be an artefact of the number of habitats sampled and thus the fit of our sampling design. For example, when our random sample included more closed habitats, nest sites were significantly more open than random (e.g. Cairns: Mossman Gorges sites). Conversely, when the nesting area was entirely open (only one habitat) we found no difference in openness between nest sites and potential nest sites (e.g. Cairns: Warraker Creek and Tully Gorge sites).

Radiation striking the top of the canopy differed considerably among our sites. For example, above-canopy radiation intensity for our *study sites*,

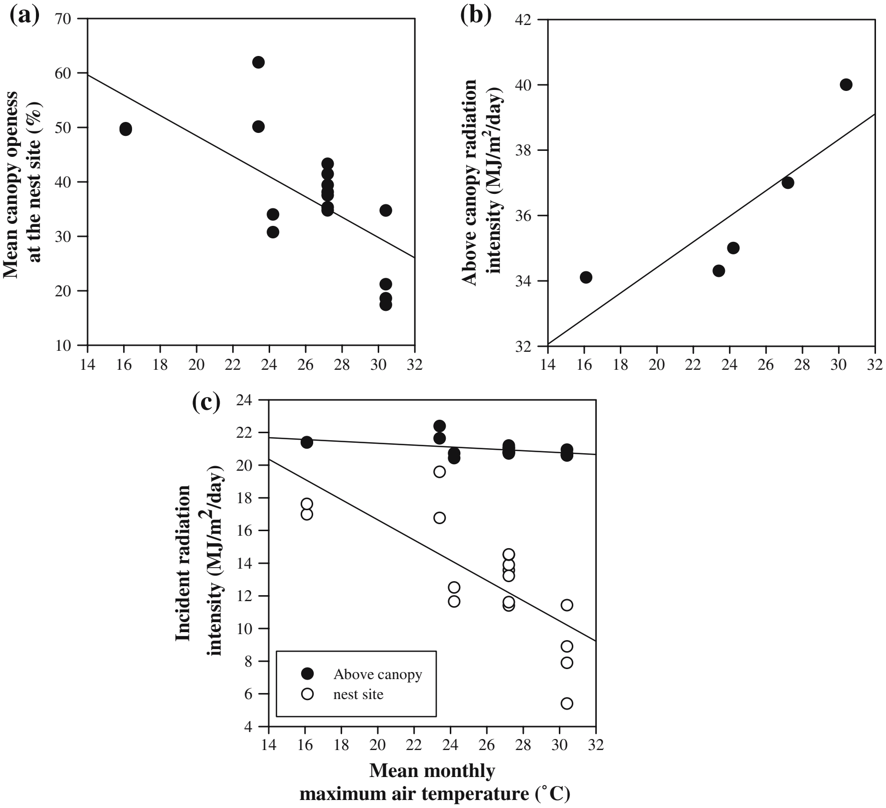

Figure 3. Relationships between mean monthly maximum temperature at the study sites and (a) mean canopy openness at nest sites; (b) above canopy radiation intensity for the study sites, measured with fixed meteorological data; and (c) incident radiation above the canopy and at the nest site, measured from the nest site.

Table 2. Comparisons of the two major nest site characteristics influencing nest temperatures between nest sites and potential nest sites (random sites)

Canopy openness (%)

Site	Locality	Nests	Random	F	p
Cairns	Mossman Gorge1	22.6 (8)	15.7 (48)	11.14	0.002**
Cairns	Mossman Gorge2	13.4 (11)	10.6 (37)	4.82	0.033*
Cairns	Tully Gorge	33.3 (7)	43.6 (50)	2.69	0.107
Cairns	Warraker Creek	14.3 (7)	19.8 (30)	1.53	0.225
Brisbane	Mt. Coot-tha BG	28.6 (4)	19.8 (43)	2.19	0.146
Brisbane	Anzac Park	30.8 (14)	23.5 (19)	5.27	0.029*
Brisbane	Chardon Bridge	42.7 (6)	44.7 (19)	0.47	0.499
Sydney	Lane Cove River	27.4 (4)	25.5 (20)	0.30	0.588
Canberra	Tuggeranong	62.6 (11)	52.0 (20)	12.23	0.002**
Canberra	ANBG	52.6 (19)	48.8 (19)	1.35	0.252
Highlands	Blue Water Holes	51.0 (6)	44.3 (20)	7.57	0.011*
Highlands	Gudgenby	51.3 (5)	31.7 (20)	12.20	0.002**
Incident radiation intensity (MJ/m^2/day)					
Cairns	Mossman Gorge1	51.2 (8)	32.5 (48)	17.18	0.0001***
Cairns	Mossman Gorge2	28.3 (11)	19.9 (37)	12.69	0.0008***
Cairns	Tully Gorge	51.7 (7)	65.0 (50)	1.91	0.173
Cairns	Warraker Creek	28.6 (7)	36.3 (50)	0.77	0.387
Brisbane	Mt. Coot-tha BG	47.2 (4)	30.8 (43)	2.10	0.154
Brisbane	Anzac Park	46.2 (14)	36.3 (19)	1.89	0.179
Brisbane	Chardon bridge	70.6 (6)	68.1 (19)	0.36	0.556
Sydney	Lane Cove River	50.9 (4)	38.2 (20)	2.74	0.112
Canberra	Tuggeranong	87.1 (11)	75.3 (20)	7.16	0.012**
Canberra	ANBG	81.1 (19)	71.0 (19)	4.43	0.042*
Highlands	Blue Water Holes	81.6 (6)	70.4 (20)	7.32	0.013*
Highlands	Gudgenby	78.4 (5)	60.1 (20)	10.10	0.004**

Data are means. Sample sizes are in parentheses. Statistics are from single-factor ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ANBG, Australian national botanic gardens; BG, botanic gardens.

calculated by Gap Light Analyser using meteorological data and controlled for season (but highlands excluded due to elevational influences), was significantly, positively related to mean monthly maximum air temperature at the sites ($r^2 = 0.99$, $F_{1,3} = 321.21$, $p = 0.003$; Fig. 3b). In contrast, above-canopy radiation intensity calculated for *nest sites* (pooled by study population), measured from the nest site was significantly inversely related to mean monthly maximum air temperature (Fig. 3c; $r^2 = 0.29$, $F_{1,16} = 6.12$, $p = 0.026$), mean monthly minimum air temperature ($r^2 = 0.62$, $F_{1,16} = 24.04$, $p < 0.0001$), and mean monthly soil temperature ($r^2 = 0.62$, $F_{1,14} = 21.24$, $p < 0.001$). These two patterns may seem contradictory, but two factors easily explain the difference. First, because lizards nested earlier in warmer climates (see below), our nests tended to have earlier lay dates at higher latitudes (i.e. tracking the onset of nesting, we started our study in Cairns in September, moved to Brisbane in

October, Canberra in November, etc.). This more than compensated for the increase in above-canopy radiation with increasing air temperatures (Fig. 3b), leading to a moderately significant inverse relationship between above-canopy radiation and air temperatures for nest sites (Fig. 3c, above-canopy radiation). Second, despite the statistical significance of the relationship between above-canopy radiation and air temperature for each nest site, the slope is actually quite gentle (Fig. 3c, above-canopy radiation); the within-site variance was very low relative to among-site variation, because nests with similar lay dates at a site experience similar above-canopy radiation intensity.

Canopy openness was a major determinant of radiation intensity at the nest site (Fig. 4; $r^2 = 0.89$, $F_{1,285} = 2269.60$, $p < 0.0001$). Radiation intensity at nest sites differed from random at some sites but not others (Table 2). The reason for this finding is the same as for our mixed results for canopy openness. Nest sites received more incident radiation than random in localities with more closed habitats, while no differences existed between nest sites and potential nest sites at localities that were entirely open or homogeneous.

Relationships among nest temperature, canopy openness and radiation of nest sites

We analysed nest temperatures in two ways: we calculated the mean daily temperature, and the mean daily range of temperatures for each day, and averaged these to examine relationships among nest temperature, canopy

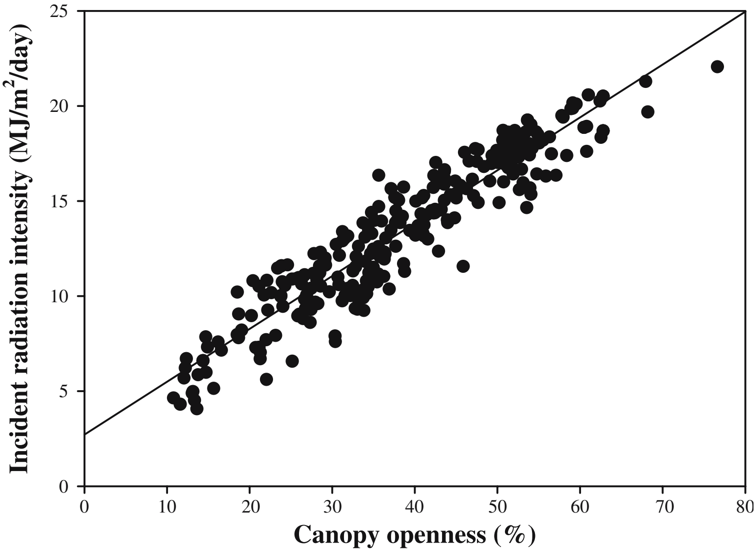

Figure 4. Relationship between incident radiation intensity and percentage canopy openness for all nest sites in the study. Data are from hemispherical photographs and Gap Light Analyser.

openness at the nest site, and incident radiation intensity at the nest site. Canopy openness and radiation intensity at the nest site were both major determinants of nest temperatures, but predominately on the mean daily range of nest temperatures. Mean daily range in nest temperatures was significantly positively related to openness and radiation intensity (Fig. 5a and b; openness: $r^2 = 0.43$, $F_{1,31} = 23.08$, $p < 0.0001$; radiation: $r^2 = 0.38$, $F_{1,31} = 18.29$, $p < 0.001$). In contrast, mean nest temperature was less significantly (positively) related to openness (Fig. 5c; $r^2 = 0.17$, $F_{1,31} = 6.08$, $p = 0.020$), and unrelated to radiation intensity at the nest site (Fig. 5d; $r^2 = 0.05$, $F_{1,31} = 1.72$, $p = 0.199$).

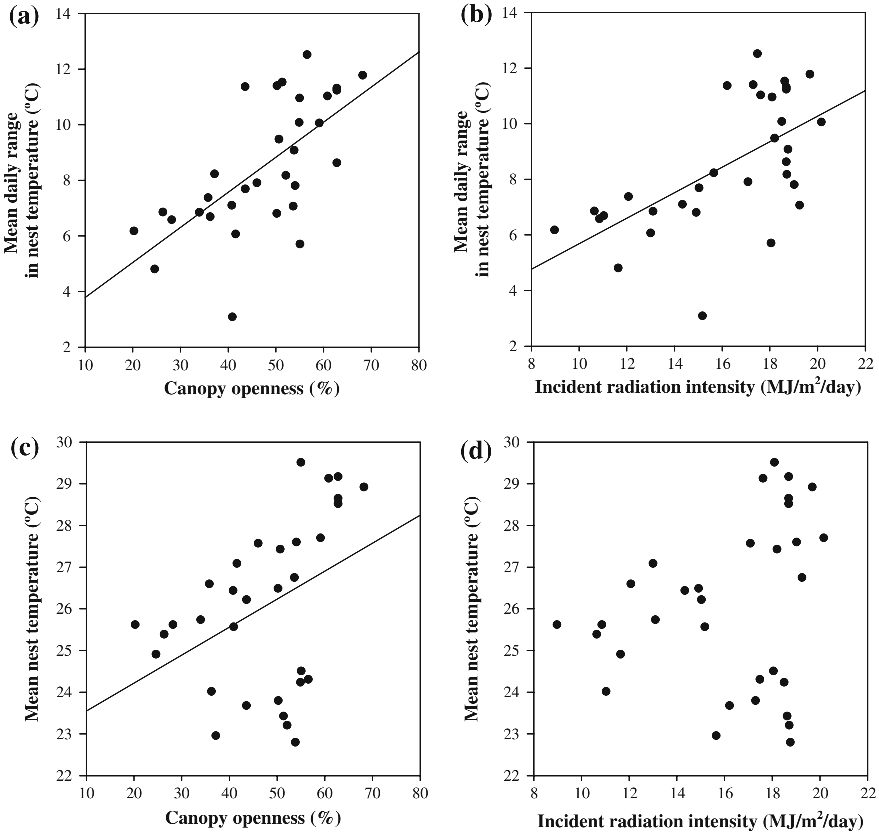

Figure 5. Relationships between two descriptors of nest temperatures (mean and mean daily range) and two measures of nest sites (canopy openness and incident radiation intensity at nest sites). Nest temperature data were continuous nest temperatures taken by data loggers at one-hour intervals throughout incubation. Canopy openness and radiation data are from hemispherical photographs and Gap Light Analyser.

Pivotal temperature experiments

We determined the sex of 583 hatchlings representing 85 clutches (Brisbane: 21 clutches, 168 eggs; Sydney: 28 clutches, 165 eggs; Canberra: 16 clutches, 114 eggs; highlands: 20 clutches, 136 eggs). Although the developmental stages at the start of incubation differed among sites (single factor ANOVA: $F_{3,83} = 4.67$, $p = 0.005$), all eggs used were younger than developmental stage 32. Our experiment confirmed that *P. lesueurii* has two pivotal temperatures, but we found no evidence of clinal variation in hatchling sex ratios (Fig. 6). Our data allowed statistical analysis of the upper pivotal temperature only. While sex ratios varied significantly with temperature at all sites (logistic regression, $T > 27$ °C, $X^2 = 189.44$, df = 1, $p < 0.0001$), there was no significant difference in the pivotal temperature across sites ($X^2 = 7.19$, df = 3, $p = 0.06$), and no trend was evident (Fig. 6). The overall relationship between sex ratio and incubation temperature yielded an estimate for the pivotal temperature of 28.0 °C (range = 27.8–28.3 °C).

Interestingly, at most sites no temperature produced 100% males (Brisbane 66%, Sydney 100%, Canberra 90%, highlands 97%). Incubation at the hotter end (30 °C) produced 100% females, but we did not achieve the low-end temperature producing 100 % females (Fig. 6). However, 25 °C produced < 50%

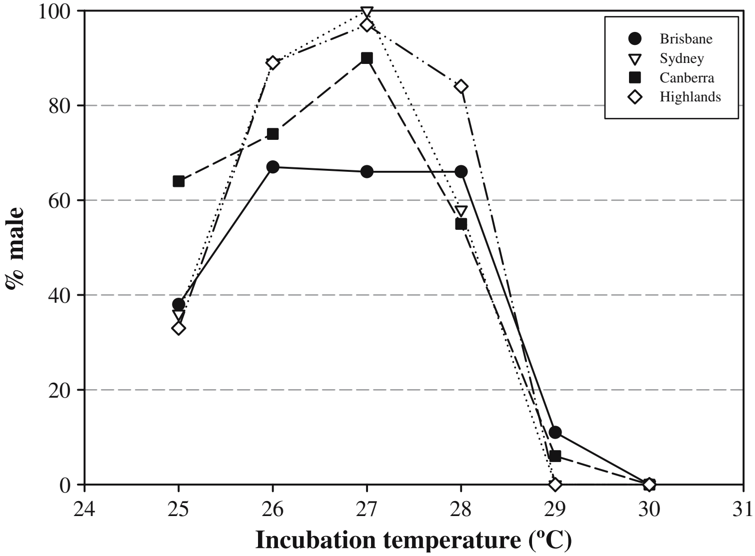

Figure 6. Geographic variation in pivotal temperatures (PT's) for *P. lesueurii*. There are two PT's, one near 25 °C and another near 28 °C. Data are from constant temperature experiments in the laboratory (= incubators set at one degree intervals between 25 and 30 °C inclusive). Eggs were not available from Cairns.

males for all sites except Canberra (64%), indicating that our experimental data were sufficient to test our hypothesis (Fig. 6).

Nesting phenology and nest depth

The onset of nesting exhibited strong clinal variation: nesting began in late September in Cairns, early October in Brisbane, mid- to late October in Sydney, early to mid-November in Canberra, and early December in the southern highlands. We also found clinal variation in the number of clutches produced. Based on nest surveys, one clutch is produced in the highlands, two clutches in Canberra, 2–3 clutches in Sydney, and at least three clutches in Brisbane. We were not able to determine clutch frequency for the Cairns populations, but at least two clutches are deposited (G. Turner, unpubl. data).

Nest depth did not exhibit clinal variation (Fig. 7). However, a significant difference in nest depths among sites was detected ($F_{3,127} = 37.95, p < 0.0001$). A Tukeys HSD post-hoc test indicated that nests in Canberra were significantly deeper ($p < 0.0001$) than those in the other three sites; no other significant differences existed among sites (all $p > 0.05$). We hypothesised that deeper nests in Canberra may have been due to a severe drought during Nov–Dec 2003 (Bureau of Meteorology, unpubl. data). To test this we obtained nest depths from the same two populations in 2004, a more typical Nov–Dec in terms of rainfall (Bureau of Meteorology, unpubl. data). However, nests depths in Canberra were similar between years ($F_{1,61} = 3.74$, $p = 0.058$; means: 2003 = 11.1 cm, 2004 = 9.9 cm), compared to other sites (e.g. Brisbane = 6.5 cm, Sydney = 7.6 cm, highlands = 6.9 cm). We were unable to obtain nest depths from a statistically valid sample of Cairns nests.

Discussion

Our study provides strong evidence for maternal nesting behaviour as a mechanism for adjusting sex ratios to maintain viable populations across environmental extremes. Air and soil temperatures exhibited marked directional variation across latitude and elevation (Figs. 1 and 2), yet nest temperatures did not (Fig. 2). Water dragons ‘compensated’ for those climatic differences by adjusting where they nested, and to a lesser extent when they nested. Canopy openness at the nest site was dramatically greater at sites with lower air temperatures (Fig. 3a). For example, the average canopy openness for the Cairns sites was 21% compared to 50% at the southern highlands sites, despite the opportunity for lizards to select sites with a range of openness at both localities. Because canopy openness was a major determinant of incident radiation intensity at the nest site (Fig. 4), radiation intensity at the nest site

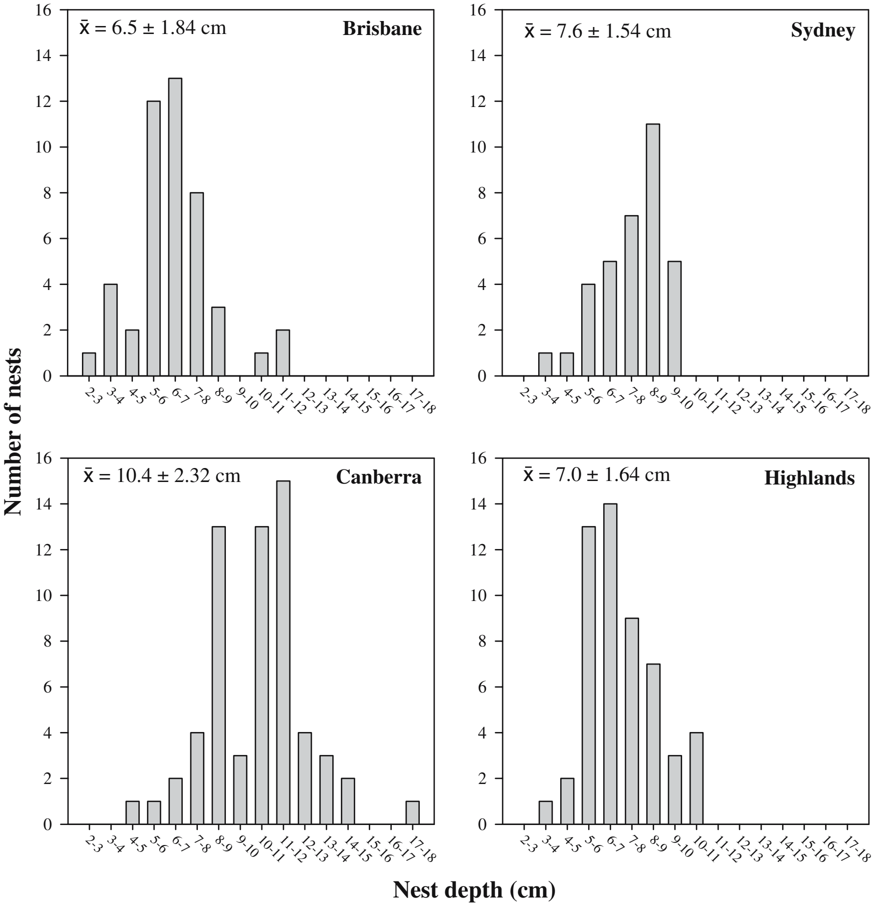

Figure 7. Geographic variation in nest depths for *P. lesueurii.* Canberra data includes data from 2004–2005, and no data were available for Cairns nests.

also showed a strong, inverse relationship with air temperatures (Fig. 3c). In this way, the adjustment of canopy openness was enough to compensate for the latitudinal bias in above-canopy radiation intensity (declining strength of the sun with longer path lengths through the atmosphere; Fig. 3b), resulting in similar nest temperatures across all sites (Fig. 2).

Clinal variation in the onset of nesting was also compensatory but to a much lesser extent. For example, although nesting began in September in the northern-most Cairns and December in the southern highlands, mean maximum and minimum air temperatures are much higher at Cairns in September than those at the southern highlands in December (Fig. 1). This suggests that adjustment in timing of nesting, although influential, is not sufficient to compensate for temperature differences across latitudes. In contrast to the compensatory effects of nest site choice and timing of nesting, we found no evidence of compensation for climate differences through pivotal temperatures or nest depth (Figs. 6 and 7).

We made three assumptions in testing our hypotheses. First, we did not determine offspring sex ratios in the field. We assumed that 'healthy' populations at our study sites are indicative of viable population sex ratios. These ratios need not be unity, but they would be expected not to deviate to maladaptive levels (Fisher, 1930; Freedberg and Wade, 2004). However, we tested the prediction that nest temperatures, our surrogate for offspring sex ratios, would not differ markedly among sites, given that nest temperature directly determines offspring sex. Second, by predicting that one or more of these traits must be adjusted to compensate for climatic effects, we assume that females are not allocating sex facultatively (Werren and Charnov, 1978; West *et al.*, 2002). However, this assumption could likely be relaxed, as we see no reason why our hypotheses and sex allocation would be mutually exclusive mechanisms for sex ratio adjustment. Finally, we acknowledge that clinal variation in maternal nesting traits may primarily reflect maximising embryonic development rather than adaptive adjustment of sex ratios (Ewert *et al.*, 1994; Morjan, 2003). Our broad question concerns how TSD populations compensate for climatic differences that would hypothetically skew sex ratios (maintenance), regardless of adaptive origin (evolution).

Our study supported the theoretical assertion that nest site choice is a target mechanism for sex ratio selection in TSD reptiles in varying or changing climates (Bulmer and Bull, 1982; Bull, 1983), and that it is more likely to be influenced by selection than pivotal temperatures, despite the predictions of simulation models (Morjan, 2003). Our data are partially consistent with the only other major study on reptiles considering both pivotal temperatures and nest site choice in a TSD species. Snapping turtles nested in more open areas at higher latitudes, but also exhibited clinal variation in pivotal temperatures (Ewert *et al.*, 2005). In that study, higher latitude pivotal temperatures were both higher and lower than lower latitude temperatures (i.e. there are two pivotal temperatures, and each was shifted away from one another incrementally with increasing latitude). Our study species also has dual pivotal temperatures, but we found no trend with latitude. This diversity of findings may indicate that sex-determining traits subject to sex ratio selection may vary among TSD species or groups. More studies on wide-ranging species are needed before any general conclusions can be made.

The few empirical studies addressing among-clutch variation in nest site choice in TSD species either detected significant differences in nest site temperatures, or found reasonably high repeatability estimates or heritabilities (Bull *et al.*, 1988; Bragg *et al.*, 2000; Janzen and Morjan, 2001). Considerable variation in openness existed among nest sites within our study sites. Although we did not measure the repeatability of these choices within individuals, we found evidence of high fidelity to nest site at several of our study sites. In many cases lizards apparently nested at the same site (within 1 m^2) as their previous nest (within years). Although we did not link mothers with nests, we consider the clumped spatial distribution of nests in pairs at many sites as circumstantial evidence of nest site fidelity. We also observed the same clumping among years (unpubl. data). Although this fidelity to nest site may indeed be related to sex production, alternatively a nesting lizard could save time and/or energy by placing her second clutch near her first clutch, or this year's clutches near last year's clutches, regardless of the influence of that choice on offspring sex.

Behavioural lability across environmental extremes

Evolutionary biologists would like to be able to predict how populations of species respond to changing environments, either in the context of climate change or the invasion of new environments at the limit of a species' distribution. Wide-ranging species are challenged with climatic differences that theoretically shape traits that directly influence lifetime reproductive success in those populations (Dunham *et al.*, 1989; Adolph and Porter, 1993; Shine, 2002). Multi-population studies can provide insight into this adaptability because we can make straightforward predictions of what attributes might be adjusted across populations (Hertz and Huey, 1981; Shine, 2002). By sampling in 'wide space' ecologists can infer evolutionary processes across 'deep time' (Brown, 1995), which assumes that variation in the driving influences such as temperature across space can provide insight into the potential to respond to climate change through time.

There are several ways in which populations can compensate for differences in climate to maintain stable populations including behavioural, physiological, and morphological responses (Hertz and Huey, 1981; Huey and Bennett, 1990; Hertz, 1992). In the mobile adult and juvenile life history stages of reptiles the primary compensatory mechanism is apparently behavioural (Hertz and Huey, 1981; Ellner and Karasov, 1993; Gvozdik, 2002; but see Hertz, 1992). While the egg stage of wide-ranging species is met with the same challenges, eggs are immobile and thus any behavioural regulation would be dependent on the mother during nest site choice (Shine *et al.*, 1997). Indeed, the immobile egg clutch is a condition that has apparently favoured the evolution of viviparity in reptiles inhabiting cold climates, because viviparous mothers can thermoregulate with the eggs (reviewed in Shine, 2002). In oviparous species, however, behavioural compensation would be restricted to the mother's choice of where, when, and how deep to nest. Our study, along with the findings of Ewert *et al.* (2005) extend to the egg stage the life history theory prediction that behavioural compensation is the chief mechanism for adjustment to among-population climatic differences, although the former study also implicated a physiological mechanism (clinal variation in pivotal temperatures). More research in other species is needed to confirm that behaviour is the primary compensatory mechanism for climatic differences experienced by the egg life history stage.

Mean vs. variance in nest temperatures

Which component of nest temperatures, the mean or the variance, is influenced by nest site characteristics chosen by reptiles? Recent studies have suggested that reptiles might choose nest sites based on the variance in nest temperatures that eggs will experience, rather than the mean temperature (Shine *et al.*, 2003; Georges *et al.*, 2004), yet quantitative data are lacking. In our study openness and incident radiation intensity at the nest site influenced nest temperatures, but in a specific way: only the daily range in nest temperature was significantly affected by both openness and incident radiation intensity (Fig. 5). Nest sites with more open canopies and therefore higher incident radiation intensity exhibited considerably higher variation in nest temperatures (Fig. 5). The influence of fluctuations in nest temperatures on sex ratios has been revealed in recent years (Georges, 1989; Georges *et al.*, 1994), and daily fluctuation in nest temperatures is at least as important as the mean temperature in determining offspring sex in shallow-nesting reptiles with TSD (Georges *et al.*, 2004). Daily fluctuations in temperature have also been shown to influence embryonic survival in lizards (Shine, 2002; Shine *et al.*, 2003), and reptilian offspring phenotypes are also sensitive to changes in the daily range of temperatures (Shine and Harlow, 1996; Shine *et al.*, 1997). Understanding which component of nest temperatures is influenced by nesting decisions could be critical to linking nest site choice, TSD, and lifetime reproductive success, and could ultimately contribute to our attempts at explaining the evolution of TSD, which has remained elusive (Shine, 1999; Valenzuela, 2004). Our study is unique in demonstrating that nest site choice influences mainly the daily range or variation in nest temperatures, rather than the mean temperatures, in oviparous reptiles.

Openness, radiation and the cues for nest site choice

At first glance our data might seem to support the notion that *P. lesueurii* simply nests in ‘open areas’ (Harlow, 2001). However, our results indicate that this classification does not adequately capture the complexity of nest site choice with respect to canopy openness in the species. Water dragons nested in areas with varying degrees of openness, and our data demonstrate consequences of those choices in the incident radiation intensity at nest sites, and nest temperatures.

What cues do nesting female *P. lesueurii* use to choose nest sites with thermal regimes that are optimal for either or both embryonic development and sex production? Although our study was not designed to determine what cues lizards use to choose a nest site with a particular thermal regime, in theory lizards could detect canopy openness visually, or detect differences in radiation or temperature by basking or by assessing substrate temperatures. These attributes were highly inter-correlated at most nest sites (Fig. 4; see also Pringle *et al.*, 2003), and disentangling them would require experimental manipulation. However, these two factors were uncoupled in our coldest population. Lizards at this site nested at the warmest aspects (unpubl. data), despite the apparent abundance of equally-open sites with cooler aspects, indicating that openness was not the cue, but rather lizards detected radiation or temperature through basking or assessing substrate temperatures, based on the path of the sun through the gorge (shading at the site is primarily by gorge walls and rocky hills). Thus, although generally canopy openness was a successful predictor of incident radiation intensity at the nest site in our study (Fig. 4), there is potential for considerable variation in radiation based on the orientation of the sun's path through canopy gaps of the same size (Pringle *et al.*, 2003).

Implications for climate warming

An imminent warming of the global climate has pervaded much of scientific thought for over two decades. Various global climate change models have predicted increases in mean air temperatures of 1–6 °C over the next 100 years (Folland *et al.*, 2001), and regional models are now available that can predict changes in temperature at the sub-continental scale (Giorgi *et al.*, 2001). For example, one model predicts an increase in mean temperature of 3–7 °C in northern Australia and 2–6 °C in southern Australia during the months Dec–Feb (Giorgi and Francisco, 2000).

Rapid climate warming is expected to have profound effects on living systems (reviewed in McCarty, 2001), including influencing their geographic distributions (Root *et al.*, 2003; Parmesan and Yohe, 2004). Increased air temperatures can influence critical parameters such as population growth rates and immigration rates (Sæther *et al.*, 2000) and the onset of reproduction (Forchammer *et al.*, 1998). Although not yet demonstrated, an even more direct influence of climate warming would be incurred by species with temperature-dependent sex determination (TSD), because offspring sex is directly and irreversibly determined by incubation temperature. This prompted Janzen (1994b) to propose that populations of TSD species may serve as ideal indicators of the biological impact of global temperature change. Wide-ranging

TSD species are faced with the likelihood of unbalanced sex ratios as the climate continues to warm (Janzen, 1992; Janzen and Paukstis, 1991; Janzen, 1994b). Populations without evolutionarily labile sex-determining traits to compensate for such warming face range retraction or fragmentation, and possibly extinction (Janzen, 1992; Janzen, 1994b; Davenport, 1997).

In the only study attempting to predict the effects of climate warming on a TSD species, Janzen (1994b) estimated that the production of male painted turtles (*Chrysemys picta*) would be effectively eliminated with a projected increase in mean air temperature of 4 °C. However, we do not know which sex-determining attribute(s) is evolutionarily labile in that species. Discovering directional or predictable clinal variation in sex-determining attributes should reveal which of those attributes reflect the species' repertoire for responding to climate change. Our multi-population approach allowed us to determine that nest site choice was adjusted across environmental extremes, and from this we hypothesise that maternal nesting behaviour is a sex-determining trait that is subject to climate-induced sex ratio selection in *P. lesueurii*, and possibly other TSD reptiles. Further studies are needed to determine whether or not such a response could occur rapidly enough in the face of current and forecasted climate warming (see similar arguments for pivotal temperatures in Janzen, 1992, 1994b; Rhen and Lang, 1998).

In summary, our study identifies maternal nesting behaviour – specifically nest site choice – as the major sex-determining trait that compensates for climatic differences among populations of *P. lesueurii* (Bulmer and Bull, 1982; Bull, 1983; Morjan, 2003; Ewert *et al.*, 2005). Timing of nesting played a minor role, but nest depth and pivotal temperatures were not implicated. More broadly, our study extends to the egg stage the conventional idea that reptile populations compensate for climatic differences mainly through behavioural means (Hertz and Huey, 1981; Gvozdik, 2002). We found that openness and radiation intensity at the nest site influenced mainly daily fluctuations in nest temperatures rather than mean nest temperatures, providing a link between nest site choice and a descriptor of nest temperatures that is a critical determinant of offspring sex ratios (Georges, 1989; Georges *et al.*, 1994), embryonic survival (Shine *et al.*, 2003), and offspring phenotypes (Shine and Harlow, 1996; Shine *et al.*, 1997). Finally, our study indicates that maternal nesting behaviour may be the chief sex-determining attribute in the repertoire of TSD species to compensate for climate warming (Janzen, 1992; 1994b). However, given the differences in our findings and those of Ewert *et al.* (2005), more research is needed to confirm the relative importance of nest site choice and pivotal temperatures in sex ratio selection of TSD species in changing climates. To this end studies including larger samples of nests and offspring sex ratios would be warranted.

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