



Nest-site selection in two eublepharid gecko species with temperature-dependent sex determination and one with genotypic sex determination

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At present, most turtles, all crocodylians, and several lizards are known to have temperature-dependent sex determination (TSD). Due to the dependence of sex determination on incubation temperature, the long-term survival of TSD species may be jeopardized by global climate changes. The current study was designed to assess the degree to which this concern is justified by examining nest-site selection in two species of Pattern II TSD geckos (*Eublepharis macularius* and *Hemitheconyx caudicinctus*) and comparing these preferences with those of a species with genotypic sex determination (GSD) (*Coleonyx mitratus*). Temperature preferences for nest sites were found to be both species-specific and female-specific. While *H. caudicinctus* females selected a mean nest-site temperature (32.4°) very close to the upper pivotal temperature (32°C) for the species, *E. macularius* females selected a mean nest-site temperature (28.7°C) well below this species' lower pivotal temperature (30.5°C). Thus, the resultant sex ratios are expected to differ between these two TSD species. Additionally, nest-site temperatures for the GSD species were significantly more variable (SE = ±0.37) than were temperatures for either of the TSD species (*E. macularius* SE = ±0.10; *H. caudicinctus* SE = ±0.17), thereby further demonstrating temperature preferences within the TSD species.

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INTRODUCTION

At present, most turtles, all crocodylians, and several lizards are known to have temperature-dependent sex determination (TSD) (Bull, 1980, 1983; Ewert & Nelson, 1991). Unlike species with genotypic sex determination (GSD), in which the sex of the offspring is determined at conception by chromosomal factors, in TSD it is the temperature at which an individual is incubated that determines its sex. Three patterns of TSD have been observed in reptiles: Patterns IA, IB, and II (Ewert & Nelson, 1991). Patterns IA and IB each have a single pivotal temperature, where *pivotal temperature* is defined as the constant incubation temperature that results in a 1:1 sex ratio (Mrosovsky & Pieau, 1991). In pattern IA, primarily males are produced below and primarily females are produced above the pivotal temperature. This pattern is reversed in Pattern IB, where primarily females are produced below and primarily males are produced above the pivotal temperature. Although Pattern IB was initially reported for several lizard species, further investigation indicates that these species actually display Pattern II TSD (Viets *et al.*, 1994). Pattern II TSD species have two pivotal temperatures, with predominantly females produced when the incubation temperature is either below the lower pivotal temperature or above the upper pivotal temperature. At temperatures between the two pivotal temperatures, predominantly males are produced.

Because the sex of TSD individuals is determined by their incubation temperatures, it has been suggested that, if the environment changes, long-term consequences of these changes could jeopardize the evolutionary stability of such species (Bull, 1980; Bulmer & Bull, 1982; Vogt & Bull, 1982). For example, a 4°C mean temperature increase, predicted by some to be likely to occur within the next 100 years (Schneider, 1989; Marabe & Stouffer, 1993), could effectively eliminate the production of male offspring in some Pattern IA TSD species and ultimately cause local extinction, as Janzen (1994) predicts for the *Chrysemys picta* (painted turtle) population in Illinois. Standora & Spotila (1985) have suggested that even a 2°C shift in incubation temperature could have dramatic effects on TSD species in only a few generations. Thus, the long-term survival of TSD species in their current distributions is dependent on their ability to adapt to environmental changes.

While long-term environmental changes could lead to sex ratio biases, Fisher (1930) predicts that selection will act on other factors, such as zygotic sex determination and maternal behaviour, to restore the sex ratio to equilibrium. The natural extension of such a prediction is that pivotal temperatures and/or maternal selection of nest-site temperatures should vary according to differences among local environments throughout a species' distribution (Bull, Vogt & McCoy, 1982).

If, in the interest of species fitness, pivotal temperatures were able to evolve in response to environmental changes, then this characteristic must be hereditary. Bull,

Vogt & Bulmer (1982) found just such a heritable component to pivotal temperatures among *Graptemys ouachitensis* (Ouachita map turtle). Thus, evolutionary changes in pivotal temperature appear to be possible in at least some TSD species. If such pivotal temperature changes were solely responsible for TSD evolution, one would expect that a species' pivotal temperature(s) would be lower in cooler, higher latitude populations than in warmer, lower latitude populations. Bull, Vogt & McCoy (1982), however, found no evidence of markedly lower pivotal temperatures in populations of *Graptemys* (map turtles), *Pseudemys* (sliders), and *Chrysemys* (painted turtles) from the northern United States when compared to populations of these species from the south, where average daily temperatures during June and July (the months of embryonic development) are 2–4°C higher. In fact, in two intraspecific comparisons, the opposite effect, a lower pivotal temperature in a lower latitude population, was found (Bull, Vogt & McCoy, 1982). Ewert, Jackson & Nelson (1994) indicate that, in addition to the trend of increasing pivotal temperatures with increasing latitude, as suggested by Bull, Vogt & McCoy (1982), there is a longitudinal trend such that, for mid-America, the overall vector for increasing pivotal temperatures in *C. picta* and *Graptomys* is from southeast to northwest.

Bull, Vogt & McCoy (1982) suggest three possible explanations for this lack of a positive association between environmental temperatures and pivotal temperatures. First, sex characteristics diagnosed in hatchlings may be irrelevant to sex in adults. Mrosovsky (1980) in turtles, Ferguson & Joanen (1982) in *Alligator mississippiensis* (American alligator), and many others, however, reported that the sex of TSD species at hatching appears to be the sex at maturity. Second, pivotal temperature in this study may not accurately reflect sex determination in nests. Bull (1985), however, studied three species of map turtles (genus *Graptemys*) and found a close association between field and laboratory studies despite the fact that natural incubation entails fluctuating temperatures and other environmental effects not incorporated in most laboratory studies. Finally, nest temperatures may not correspond to climatic temperatures, possibly because differences in nesting behaviour compensate for climatic differences.

Indeed, Ewert, Jackson & Nelson (1994) suggest that TSD, freshwater, turtle species in the southeast often choose shaded nesting sites while species in the northwest seek warm, open sites (data summarized in Ewert, Jackson & Nelson, 1994). Thus, although counter-intuitive, the trend of increasing pivotal temperatures from southeast to northwest in mid-America does reflect climatic conditions since nest-site temperatures increase from southeast to northwest, unlike ambient temperatures which decrease along the same vector. Hence, although it appears that pivotal temperatures can vary and reflect regional nesting temperatures, nesting behaviour may account for the differences between nest-site temperatures and climatic temperatures. This conclusion emphasizes the need for further examination of maternal nest-site selection.

If maternal nest-site selection is to occur in TSD species, then females must be able to distinguish between various environmental temperatures. While Janzen (1994) alluded to an absence of preferential nesting in *C. picta*, Rand (1972) found that temperatures of natural *Iguana iguana* (iguana) nests were between 31–32°C, a narrow temperature range given the wider range of soil temperatures available for nesting sites. Furthermore, Stoneburner & Richardson (1981) determined that gravid *Caretta caretta* (loggerhead turtle) move away from the ocean, up the beach, and consistently lay their eggs immediately after encountering an abrupt rise in sand

temperature of at least 2°C within 0.5 m. The results of these studies suggest that temperature is an important determinant of nesting site for at least some TSD species. Therefore, it is possible that TSD females can detect temperature differences and select preferred temperatures at which to lay their eggs. However, in the field, it is possible that some cue other than temperature is also being used to determine nest-sites. Thus, a controlled, laboratory study would be required to determine specifically whether TSD females preferentially select certain temperatures at which to lay their eggs or whether they use some other cue.

In the only known laboratory-controlled study of possible preferential nesting by a TSD species, Bull, Gutzke & Bulmer (1988) determined that *Eublepharis macularius* (leopard gecko) preferentially lay eggs at sites with intermediate temperatures (approximately 29°C) when provided the full range of viable incubation temperatures (25°C to 35°C). In order to supplement this currently limited information, the present study was designed to examine nest-site selection in three Eublepharid geckos: *Eublepharis macularius* and *Hemitheconyx caudicinctus* (African fat-tail gecko), two species of Pattern II TSD geckos (Viets *et al.*, 1993, 1994), and *Coleonyx mitratus* (banded gecko), a GSD species (Viets, 1996).

Based on Bull, Gutzke & Bulmer (1988), it was hypothesized that TSD females would preferentially select intermediate nest-site temperatures at which to lay eggs. For *E. macularius* (TSD), the complete viable range of incubation temperatures is known (25°C to 35°C) (Viets *et al.*, 1993). Therefore, an intermediate nesting temperature of approximately 29°C was expected (Bull, Gutzke & Bulmer, 1988). For *H. caudicinctus* (TSD), however, the full range of viable temperatures is not known. Thus, an intermediate temperature for this species could not be predicted. *C. mitratus* (GSD) were not expected to preferentially select intermediate nesting temperatures at which to lay eggs, since this species does not rely on temperature for sex determination. It was further hypothesized that variation would exist within species, allowing for potential adaptation to environmental changes.

MATERIAL AND METHODS

Forty animals from three species were used in this study, of which 26 were TSD individuals. Fourteen of these TSD individuals were laboratory-raised *E. macularius* (nine females and five males) and 12 were laboratory-raised *H. caudicinctus* (eight females and four males), each incubated at a constant temperature. Additionally, 14 GSD, wild-caught *C. mitratus* were used (eight females and six males).

Most of the geckos used in this study were housed as trios, consisting of two females and one male. The remainder were housed as pairs: one male and one female. These housing arrangements and sex ratios had been established prior to this study. *H. caudicinctus* and *E. macularius* adults were maintained in a room with a 12-hour light and 12-hour dark cycle and a constant air temperature of 28°C. Each TSD trio/pair was housed in a large Sterilite® box (39.5 × 27.0 × 14.0 cm) in which a water dish (9.3 cm diameter × 1.8 cm deep) and shelter were placed. The shelter was constructed from a 473 ml plastic cup (Viets, 1993). Adult *E. macularius* and *H. caudicinctus* individuals were fed 4–6 week old crickets once per week and neonatal mice twice per week, *ad libitum*.

C. mitratus were also maintained in a 12-hour light and 12-hour dark cycle, but

at a temperature of 25°C. Due to the much smaller size of this species, each trio/pair was housed in a smaller Rubbermaid® box (32.0 × 19.0 × 9.0 cm) supplied with a small water dish (6.0 cm diameter × 1.5 cm deep) and a shelter constructed from a 266 ml plastic cup (Viets, 1993). Throughout the experiment, *C. mitratus* adults were fed small (3–4 week old) crickets three times per week *ad libitum*. Neonatal mice were not fed to *C. mitratus* due to the small size of this gecko species.

Calcium carbonate was added to the food of all species twice per week and vitamin supplements were provided once per week. In addition, water was provided to all individuals *ad libitum*. For further details of all procedures, see Bragg (1996).

All females of each species were examined weekly to determine the stage of egg development. When a female was considered to be gravid, she was removed from the housing chamber and isolated in a nesting chamber. All females were in a nesting chamber for at least 12 hours prior to egg deposition to allow for an acclimation period to the provided temperature gradient.

Each nesting chamber consisted of a 39.5 × 27.0 × 14.0 cm Sterilite® box filled with 3–4 cm of lightly packed vermiculite (1:1.5 vermiculite to water, by mass). The vermiculite was misted and remixed daily to help maintain the original 1:1.5 ratio of vermiculite to water. Each chamber contained one large water dish (9.3 cm diameter × 1.8 cm deep) and two large shelters (473 ml cup cut lengthwise). To ensure that nest-site selection was not based on the location of shelters, three different shelter arrangements were used (Bragg, 1996). Each female was exposed to each shelter arrangement at least once and was rotated through the three arrangements throughout the breeding season.

A temperature gradient was maintained across each nesting chamber using procedures established by Viets (1993) and modified by Bragg (1996). For *E. macularius* and *H. caudicinctus* the thermal gradient provided for egg-laying ranged from 35 ± 2°C to 28 ± 1°C. While eggs of these species may be able to hatch above (e.g. for *H. caudicinctus*) and below (e.g. for *E. macularius*) this range, in this study, *E. macularius* and *H. caudicinctus* were both housed in the same room and under the same conditions, thus the same temperature gradient was used for both of these TSD species. The lower gradient temperature was set at 28°C since *H. caudicinctus* eggs are not viable below this temperature (Viets *et al.*, 1994). The upper gradient limit was set at 35°C since *E. macularius* eggs are not viable above this temperature (Viets *et al.*, 1993). For *C. mitratus*, the GSD species, the thermal gradient ranged from 33 ± 1°C to 24 ± 1°C which represents the full range of viable incubation temperatures for this species (Viets, unpublished data). Each gradient was checked twice daily and adjusted as necessary to maintain a constant temperature gradient.

Nesting chambers were examined twice daily for eggs. If it was determined that a female had laid her eggs, the date and time were noted and the female was weighed to record post-oviposition weight (POW). The female was then returned to her regular housing chamber with the appropriate cagemates. Upon locating a clutch, a thermistor (accurate to ±0.1°C) was immediately used to measure the temperature of the vermiculite directly under the newly-deposited eggs. Although ultimately not used in analyses, other information was collected including the depth to which an egg had been buried and a sketch of the cage indicating the gradient, location of the shelters and water dish, and location and egg number(s) of the egg(s) found (Bragg, 1996). Once this information was recorded, the eggs were removed from the nesting chamber, weighed, and candled (Ewert, 1985; Hubert, 1985).

The effect of clutch parameters on nest-site temperatures was tested by ANCOVA

TABLE 1. ANCOVA run on all species combined with timing within the laying season, post-oviposition weight (POW), an egg weight as covariates and with species and shelter arrangement as the treatment effects. Significant values are indicated with an *

Source:	DF	Type III sum of Squares	F-value	P-value	Sample size
Timing within laying season	1	60.38	21.63	0.0001*	283
POW	1	0.89	0.32	0.5734	283
Egg weight	1	0.47	0.17	0.6819	283
Species	2	875.08	156.72	0.0001*	283
Shelter arrangement	2	6.31	1.13	0.3243	283
Species-shelter interaction	4	13.49	1.21	0.3077	283

TABLE 2. Mean, SE and range of nest-site temperatures for each species

Species	Mean and standard error	Range		Sample size
		Min	Max	
<i>E. macularius</i>	28.7 ± 0.10	26.9	33.0	131
<i>H. caudicinctus</i>	32.4 ± 0.17	27.7	36.9	98
<i>C. mitratus</i>	27.8 ± 0.37	23.6	34.1	54

(SAS Institute, 1987). Timing within the laying season, post-oviposition weight (POW) of the female, and egg weight were the covariates, while species, female, and shelter arrangement were the treatment effects. Both the Student–Newman–Keuls and the Scheffe tests were applied using a Type III Sum of Squares to assess significance. Both of these tests gave similar results. *P*-values reported in the test are based on Scheffe tests. Differences were considered significant at $P \leq 0.05$.

RESULTS

E. macularius and *H. caudicinctus* breed seasonally. During the 1995–1996 breeding season, *E. macularius* laid eggs between 7 December 1995 and 9 July 1996, and *H. caudicinctus* laid eggs between 9 January and 14 June 1996. In contrast, *C. mitratus* breeds intermittently year-round in captivity. Due to space and time constraints, clutches from *C. mitratus* were collected only from 20 January to 8 July 1996.

Of the total of 170 clutches, 17 were unburied. In the case of unburied fertile clutches ($n=9$), nest excavation was interrupted, while unburied, infertile clutches ($n=8$) were simply deposited on an undisturbed vermiculite surface. All unburied clutches, regardless of fertility, were excluded from the data used for analysis due to the potential influence of ambient temperature on recorded nest-site temperatures. Through the breeding season, 10 completely infertile clutches were laid, eight were unburied and two were buried. Only buried clutches that contained at least one fertile egg ($n=151$ clutches or $n=283$ eggs) were used in the analyses of these data.

An ANCOVA with species and shelter arrangement as treatment effects and with timing within the laying season, POW, and egg weight as covariates was run to determine the effects of these factors on nest-site temperature. Species ($P=0.0001$) and timing within the laying season ($P=0.0001$) were the only significant factors identified in this test (Table 1). The mean nest-site temperatures for each species are listed in Table 2.

TABLE 3. ANCOVA run by species with timing within the laying season, post-oviposition weight (POW), and egg weight as covariates and with female and shelter arrangement as treatment effects. Significant values are indicated by *

Species	Source	DF	Type III sum of squares	F-value	P-value	Sample size
<i>E. macularius</i>	Timing within laying season	1	19.06	18.77	0.0001*	131
	POW	1	9.28	9.14	0.0032*	131
	Egg weight	1	9.45	9.30	0.0029*	131
	Female	8	38.45	4.73	0.0001*	131
	Shelter arrangement	2	3.75	1.85	0.1631	131
	Female-shelter interaction	16	29.29	1.80	0.0407*	131
<i>H. caudicinctus</i>	Timing within laying season	1	13.39	7.36	0.0073*	98
	POW	1	1.31	0.75	0.3899	98
	Egg weight	1	0.37	0.21	0.6485	98
	Female	7	57.60	4.69	0.0002*	98
	Shelter arrangement	2	14.23	4.06	0.0215*	98
	Female-shelter interaction	14	61.45	2.50	0.0060*	98
<i>C. mitratus</i>	Timing within laying season	1	3.02	0.79	0.3813	54
	POW	1	2.24	0.58	0.4506	54
	Egg weight	1	1.04	0.27	0.6055	54
	Female	7	119.53	4.46	0.0017*	54
	Shelter arrangement	2	4.05	0.53	0.5949	54
	Female-shelter interaction	11	28.80	0.68	0.7428	54

Since species was a significant treatment effect in the species-wide test (Table 1), the data were sorted by species. An ANCOVA with shelter arrangement and female as treatment effects and with timing within the laying season, POW, and egg weight as the covariates was then run to determine the effects of these factors on the selection of nest-site temperature (Table 3). The mean nest-site temperatures for each individual female within each species are listed in Table 4.

Since timing within the laying season was considered an important covariate (Tables 1 and 3), a Pearson correlation between timing within the laying season and nest-site temperatures was conducted. The data for this test were sorted by female within each species because female had a significant effect on nest-site temperature in all three species (Table 3). From this analysis, 2 of 9 *E. macularius*, 5 of 8 *H. caudicinctus*, and 5 of 8 *C. mitratus* females showed a significant correlation between timing within the laying season and nest-site temperature (Table 5). While nearly all of these significant correlations were positive, one of the *C. mitratus* females was found to have a significant negative correlation between timing within the laying season and nest-site temperature (Table 5).

DISCUSSION

Two principal conclusions result from this study, one addressing nest-site selection by species and the other addressing selection by individuals within species. In addition, this study provides sufficient information to discuss the ultimate cause of nest-site temperature selection.

TABLE 4. Mean, SE and range of nest-site temperatures for each female within species

Species	Mean and standard error	Range		Sample size
		Minimum	Maximum	
<i>E. macularius</i>	28.8 ± 0.25	27.6	30.7	15
	29.5 ± 0.42	27.7	33.0	18
	28.2 ± 0.06	27.6	28.5	15
	28.7 ± 0.23	27.6	30.4	16
	28.6 ± 0.35	26.9	32.6	20
	29.1 ± 0.35	27.4	30.9	12
	28.7 ± 0.15	28.0	30.7	20
	28.3 ± 0.24	27.3	29.4	11
	29.1 ± 0.35	28.3	29.7	4
<i>H. caudicinctus</i>	32.4 ± 0.66	28.9	36.9	14
	32.7 ± 0.60	30.1	36.3	12
	32.4 ± 0.25	31.5	33.1	8
	31.7 ± 0.71	27.7	34.7	11
	32.5 ± 0.33	30.6	34.1	12
	31.4 ± 0.25	30.1	33.0	13
	32.5 ± 0.39	30.5	36.0	16
	33.4 ± 0.31	32.2	35.7	12
<i>C. mitratus</i>	27.4 ± 0.60	25.2	29.6	7
	26.7 ± 0.52	25.0	29.0	8
	24.0 ± —	24.0	24.0	1
	30.2 ± 0.45	28.6	32.3	8
	26.0 ± 0.54	24.9	27.0	4
	25.2 ± 0.51	23.6	27.8	9
	30.2 ± 0.73	27.4	33.7	9
	28.6 ± 1.25	24.3	34.1	8

Effects by species

The first of the two principal conclusions of this study was that selection for nest-site temperature was significantly influenced by species. It was initially hypothesized that *E. macularius* and *H. caudicinctus*, the TSD species, would preferentially select intermediate nest-site temperatures, whereas *C. mitratus*, the GSD species, would not. As noted in Bull, Gutzke & Bulmer (1988), 29°C is considered intermediate compared to the viable range of 25°C to 35°C for *E. macularius*. Because the current study could not provide nesting *E. macularius* females with the full range of temperatures provided by Bull, Gutzke & Bulmer (1988), the mean 29°C nest-site temperature termed 'intermediate' in their study was considered low in the current study. The mean nest-site temperature of 28.7°C and the small standard error ($\pm 0.10^\circ\text{C}$) noted for *E. macularius* in the current study (Table 2) support the hypothesis that *E. macularius* females preferentially select 'intermediate' (29°C) nest-site temperatures.

Results for *H. caudicinctus*, however, are less clear with respect to selecting an intermediate temperature since the upper viable incubation temperature for this species is not yet known (Viets, personal observation). To best evaluate *H. caudicinctus* in a manner similar to that used with *E. macularius*, females must be provided with a wider range of nest-site temperatures than those used in this study. Despite this limitation, the mean nest-site temperature for *H. caudicinctus* (32.4°C) was within the known viable temperature range of the species. Since *H. caudicinctus* seems to

TABLE 5. Correlation of timing within the laying season and nest-site temperature by female within each species. Significant values are indicated by *

Species	Sample size	P-value	r-value
<i>E. macularius</i>	15	0.5657	-0.16131
	18	0.0723	+0.43356
	15	0.7727	+0.08153
	16	0.5691	+0.15397
	20	0.0006*	+0.70038
	12	0.6966	-0.12593
	20	0.0156*	+0.53251
	11	0.1984	+0.42002
	4	0.1435	+0.85648
<i>H. caudicinctus</i>	14	0.0005*	+0.80315
	12	0.0197*	+0.65945
	8	0.5543	+0.24764
	11	0.0184*	+0.69166
	12	0.1615	-0.43131
	13	0.3458	+0.28467
	16	0.0463*	+0.50448
	12	0.0074*	+0.72692
	<i>C. mitratus</i>	7	0.0396*
8		0.0017*	+0.91083
1		—	—
8		0.1157	-0.60024
4		0.2773	+0.72273
9		0.0057*	+0.82958
9		0.0274*	+0.72406
8		0.0072*	+0.85214

have a viable temperature range well above that for *E. macularius*, this preferred temperature may well prove to be intermediate when compared to the full range of viable incubation temperatures, when they become known. The small standard error ($\pm 0.17^\circ\text{C}$), similar to, but significantly larger than *E. macularius* ($P \leq 0.001$, Tukey-type multiple-comparison test for differences among variances; Zar, 1984), further indicates a preference for a particular temperature.

C. mitratus females (GSD) selected a mean nest-site temperature of 27.8°C , similar to the mean temperature selected by the *E. macularius* (TSD). This temperature is indeed intermediate within the viable incubation temperature range of $24\text{--}33^\circ\text{C}$. However, the standard error (± 0.37) for *C. mitratus* was both substantially larger and significantly different than either *E. macularius* or *H. caudicinctus* ($P \leq 0.001$, Tukey-type multiple-comparison test for differences among variances; Zar, 1984), indicating less specificity in selecting a particular temperature, whether it be intermediate or otherwise. This apparent lack of nest-site temperature preference by the GSD species can also be examined by visually comparing the distribution of nest-site temperatures for the three species. While the *E. macularius* and *H. caudicinctus* nest-site temperatures are relatively normally distributed, *C. mitratus* distribution of nest-site temperatures is more platykurtic (Fig. 1).

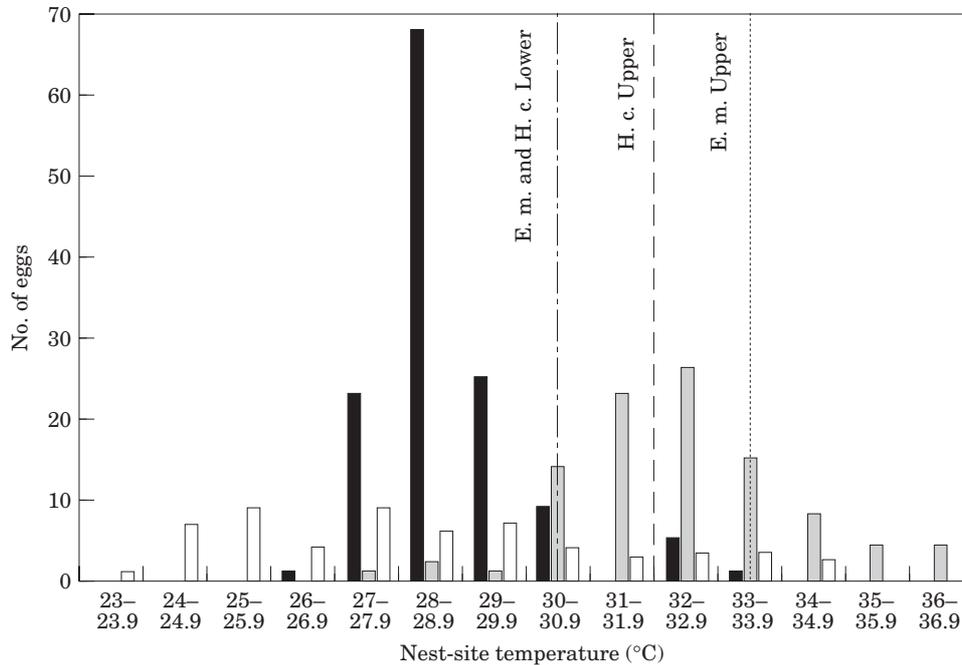


Figure 1. Distribution of nest-site temperatures for each species (■) *E. macularius*; (□) *H. caudicinctus*; (▒) *C. mitratus*. Upper and lower pivotal temperatures of the two TSD species are indicated by vertical, dashed lines. E.m. = *Eublepharis macularius*, H.c. = *Hemiteconyx caudicinctus*.

Differences in the degree of selectivity of *E. macularius*, *H. caudicinctus*, and *C. mitratus*, as represented in different standard errors for TSD and GSD species, may be explained by at least two relationships. First, GSD species may simply be genetically less selective of nest-site temperatures than TSD species, a logical explanation since GSD species are not dependent on temperature for sex determination. However, it is also possible that the variability of the incubation temperature of the adult, breeding females may affect nest-site selectivity. As noted earlier, the TSD females were incubated at constant, laboratory-controlled temperatures, while *C. mitratus* (GSD) females were wild-caught. Thus, incubation temperatures for the GSD species are likely to have been more variable than those for the TSD species, which may account for the greater variation in GSD nest-site selection found in this study. A study using GSD species from known and constant incubation temperatures would be necessary to more accurately explain differences in the variances in nest-site temperatures between the TSD and GSD species.

To further assess the differences between the two TSD species, both for evolutionary implications, and to understand future reproductive success, it is important to examine the location of selected nest-site temperatures relative to the species' pivotal temperatures. Pattern II TSD species, such as *E. macularius* and *H. caudicinctus* have two pivotal temperatures. At incubation temperatures above the highest and below the lowest pivotal temperatures, predominantly females are produced. At temperatures between the two pivotal temperatures, predominantly males are produced. For *E. macularius*, the pivotal temperatures are approximately 30.5°C and

33.5°C, and for *H. caudicinctus*, they are approximately 30.5°C and 32°C (Viets *et al.*, 1994). The mean *E. macularius* nest-site temperature in this study (28.7°C) was well below the lower pivotal temperature (30.5°C) for the species. Thus, if the temperature preferences found in this study and the pivotal temperatures determined in the laboratory apply to natural populations, one may anticipate a population sex ratio highly skewed toward females in *E. macularius*. Alternatively, *H. caudicinctus* females preferred a mean temperature (32.4°C) only slightly above their 32°C upper pivotal temperature, a temperature which produces a 1:1 sex ratio in the laboratory (Viets *et al.*, 1994). Thus, a sex ratio close to 1:1 would be expected in natural populations of *H. caudicinctus*, assuming that laboratory and field temperature preferences are comparable.

Effects by individual females

The second main result of this study is that nest-site temperature was female-specific. Contrary to the results of the study by Bull, Gutzke & Bulmer (1988), significant differences among females within all three species were found in this study: *E. macularius* ($P=0.0001$), *H. caudicinctus* ($P=0.0002$), and *C. mitratus* ($P=0.0017$) (Table 3). The different results of these two studies may be due to the fact that females in the current study were isolated to ensure that each clutch could be accurately attributed to the correct female. In contrast, Bull, Gutzke & Bulmer (1988) housed several females together, allowing for the possibility that clutches may have been incorrectly assigned to females, which would have masked individual differences. That nest-site temperature was greatly affected by individual female is of particular importance to long-term species fitness. While the differences between females do not appear large (Table 4), it is precisely these differences that may allow TSD populations to adapt to changes in environmental conditions.

Initially in this study, we asked whether the incubation temperature of a particular female would impact her selection of nest sites for her eggs. For example, O'Steen (1998) found a negative correlation in TSD *Chelydra serpentina* (snapping turtle) between egg temperature and juvenile environmental temperature choice. Similarly, we hypothesized that preferences for nest-site temperatures among adults may vary based on the adult's incubation temperature as an embryo. However, due to severely limited sample sizes (in one case a single individual represented an entire class), no statistical analyses could be done on the data obtained in the current study. However, we encourage future examination of the role of incubation temperature on adult nest-site selectivity in order to more accurately assess the origin of female nest-site temperature preferences.

Ultimate cause of nest-site selection

It has been suggested that nest-site temperatures are selected to influence either the sex ratio of offspring or the survivability of eggs. Schwarzkopf & Brooks (1987), for example, suggested that, at least in higher-latitude environments, if eggs frequently fail to complete development, then the distribution of the nest temperatures should be unimodal and close to the maximum available temperature. They further suggested that, based on Bulmer & Bull (1982), if females were selecting nest sites

to influence the sex ratio of the offspring, the distribution of nest-site temperatures would not be unimodal, but rather bimodal. While this theory is based on Pattern IA species, the idea of modality should still hold for Pattern II species. Additionally, while this theory is based on TSD species from higher latitude environments, the logic behind the modality of nest-site temperatures should apply to lower latitude species as well. Based on this rationale and the unimodality of nest-site temperatures within the two TSD species in this study (Fig. 1), one could conclude that females of these species are probably not selecting nest sites to influence sex ratios of their offspring. Rather, it is suggested that females may be selecting nest-site temperatures to ensure embryo survival.

Additionally, while addressing embryo survival to hatching, Vogt & Bull (1982) hypothesized that, at least in a temperate climate where cool temperatures abruptly terminate the growing season (e.g. southern Wisconsin, U.S.A.), females may select sunny over shaded sites (where soil temperatures at nest depth would be cooler in shaded areas than in sunny areas) at which to lay later clutches than were selected for early in the season. This hypothesis is based on the facts that (1) nests laid later in the laying season have less time to hatch before the end of the nesting season than do early nests, and (2) warmer incubation temperatures decrease the time to hatching, as in *E. macularius* (Viets *et al.*, 1993). Data from the current study indicates that females may be doing just that. Since this question was not the central focus of the present study, sample sizes are limited. Nevertheless, there are some significant, positive correlations between nest-site temperature and timing within the laying season (Table 5) that indicate that females may be selecting temperatures to ensure the survival of offspring to hatching. Studies with larger sample sizes than those available in this study, however, are necessary to determine if these are sound results or simply artifacts of incomplete data.

Several facts further complicate any conclusion that females are selecting nest-site temperatures to ensure embryo survival. For instance, it is possible that a unimodal distribution may result even if females are selecting nest sites to influence sex ratio. *H. caudicinctus*, for example, selected nest-site temperatures that were unimodally distributed with a mean very close to the upper pivotal temperature for this species (Fig. 1). Eggs incubated at these temperatures should produce a sex ratio very close to 1:1. Thus, the modality of selected nest-site temperatures, in and of itself, is not indicative of the ultimate cause of temperature selection.

Secondly, it is possible that a 1:1 sex ratio is not preferentially selected for in all TSD species. For example, the mean nest-site temperature for *E. macularius* was well below the lower pivotal temperature. Thus, it appears that this species is selecting temperatures that will strongly skew the sex ratio toward females. This may occur for at least two reasons. First, in a polygamous population, where one male can mate with several females, it is possible that fitness is favoured by a sex ratio skewed toward females. However, in such a female-dominated population, a single male would contribute more to future generations than any one female. Thus, it has been argued that long-term selection in populations with such characteristics would eventually cause the sex ratio to return to 1:1 (Fisher, 1930). Alternatively, if either the embryonic and/or post-hatching mortality of one sex of offspring is higher than that for the other sex (e.g. Burger & Zappalorti, 1988), it is possible that selection may act to favour an initially skewed population sex ratio to balance the skewed mortality ratio.

The final complicating factor to determining the ultimate cause of nest-site temperature selection is that, as previously indicated, the opposite sex is occasionally produced within 'female-producing' and 'male-producing' temperature ranges. These temperature ranges, thus, are not absolute determinants of sex. For example, Bull, Vogt & Bulmer (1982) found a heritable component to the pivotal temperature among eggs incubated at the same, constant temperature. Additionally, Viets (unpublished data) found that, while one female's eggs may all develop into female offspring at a certain temperature, another female's eggs may all develop into male offspring at the same temperature. Moreover, pivotal temperatures are known to change slightly from year to year (Viets, 1993). To account for these possibilities, a large-scale and complex study would be necessary.

Because of these several alternate hypotheses, all supported to some degree by data, there remains no definitive conclusion as to the ultimate cause of nest-site temperature selection. However, this study clearly shows that some such selection does occur, at least for the species evaluated.

CONCLUSIONS

The data in this study indicate both species-specific and female-specific differences in nest-site selection. The two TSD species, *E. macularius* and *H. caudicinctus*, both selected more tightly clustered nest-site temperatures than did *C. mitratus*, a GSD species, suggesting greater nest-site selectivity by the TSD species. Additionally, there were differences between TSD species. For example, since the mean nest-site temperature for *E. macularius* was well below its lower pivotal temperature, a sex ratio highly skewed toward females is expected in this species. In contrast, the mean nest-site temperature for *H. caudicinctus* was close to the upper pivotal temperature and should produce a near 1:1 sex ratio. In addition to species specific differences, there were differences among females of the same species. This female-specific effect was noted in all species, both TSD and GSD. Thus, the combination of intraspecific variation and the ability of females to select nest-site temperatures, should allow the TSD species considered in this study to adapt to global changes in temperature, although each in a slightly different manner.

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REFERENCES

- Bragg WB. 1996.** Nest-site selection in two gecko species with temperature-dependent sex determination and one with genotypic sex determination. Unpublished M.A. Thesis, University of Nebraska at Omaha.

- Bull JJ.** 1980. Sex determination in reptiles. *Quarterly Review of Biology* **55**: 3–21.
- Bull JJ.** 1983. *Evolution of sex determining mechanisms*. Menlo Park: Benjamin/Cummings Publishing Co., Inc.
- Bull JJ.** 1985. Sex ratio and nest temperature in turtles: comparing field and laboratory data. *Ecology* **66**: 1115–1122.
- Bull JJ, Gutzke WHN, Bulmer MG.** 1988. Nest choice in a captive lizard with temperature-dependent sex determination. *Journal of Evolutionary Biology* **2**: 177–184.
- Bull JJ, Vogt RC, Bulmer MG.** 1982. Heritability of sex ratio in turtles with environmental sex determination. *Evolution* **36**: 333–341.
- Bull JJ, Vogt RC, McCoy CJ.** 1982. Sex determining temperatures in turtles: a geographic comparison. *Evolution* **36**: 326–332.
- Bulmer MG, Bull JJ.** 1982. Models of polygenic sex determination and sex ratio control. *Evolution* **36**: 13–26.
- Burger J, Zappalorti RT.** 1988. Effects of incubation temperature on sex ratios in pine snakes: differential vulnerability of males and females. *American Naturalist* **132**: 492–505.
- Ewert MA.** 1985. Embryology of turtles. In: Gans C, Billet F, Maderson PFA, eds. *Biology of the Reptilia*. Volume 14. New York: John Wiley & Sons, 75–268.
- Ewert MA, Nelson CE.** 1991. Sex determination in turtles: diverse patterns and some possible adaptive values. *Copeia* **1991**: 50–69.
- Ewert MA, Jackson DR, Nelson CE.** 1994. Patterns of temperature-dependent sex determination in turtles. *Journal of Experimental Zoology* **270**: 3–15.
- Ferguson MWJ, Joanen T.** 1982. Temperature of egg incubation determines sex in *Alligator mississippiensis*. *Nature* **296**: 850–853.
- Fisher RA.** 1930 (1958 reprint). *The genetical theory of natural selection*. New York: Dover Publications, Inc.
- Hubert J.** 1985. Embryology of the squamata. In: Gans C, Huey RB, eds. *Biology of the Reptilia*. Volume 15. New York: Alan R. Liss, Inc, 1–34.
- Janzen FJ.** 1994. Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Sciences* **91**: 7487–7490.
- Manabe S, Stouffer RJ.** 1993. Century-scale effects of increased atmospheric CO₂ on the ocean-atmosphere system. *Nature* **364**: 215–218.
- Mrosovsky N.** 1980. Thermal biology of sea turtles. *American Zoologist* **20**: 531–547.
- Mrosovsky N, Pieau C.** 1991. Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. *Amphibia-Reptilia* **12**: 169–179.
- O'Steen S.** 1998. Embryonic temperature influences juvenile temperature choice and growth rate in snapping turtles *Chelydra serpentina*. *Journal of Experimental Biology* **201**: 439–449.
- Rand AS.** 1972. The temperatures of iguana nests and their relation to incubation optima and to nesting sites and season. *Herpetologica* **28**: 252–253.
- SAS Institute.** 1987. *SAS/STAT User's guide for personal computers*. 1987 edition. Cary: SAS Institute.
- Schneider SH.** 1989. The greenhouse effect: science and policy. *Science* **243**: 771–781.
- Schwarzkopf L, Brooks RJ.** 1987. Nest-site selection and offspring sex ratio in painted turtles, *Chrysemys picta*. *Copeia* **1987**: 53–61.
- Standora EA, Spotila JR.** 1985. Temperature dependent sex determination in sea turtles. *Copeia* **1985**: 711–722.
- Stoneburner DL, Richardson JI.** 1981. Observations on the role of temperature in loggerhead turtle nest site selection. *Copeia* **1981**: 238–241.
- Viets BE.** 1993. Lizard reproductive ecology: sex determination and parental investment. Unpublished Ph.D. Dissertation, Indiana University.
- Viets BE.** 1996. Sexual dimorphism and sex-determining mechanisms in eublepharid geckos. Abstract. Program Notes and Abstracts of the 39th Annual Meeting of the Society for the Study of Amphibians and Reptiles. Lawrence, Kansas.
- Viets BE, Ewert MA, Talent LG, Nelson CE.** 1994. Sex-determining mechanisms in squamate reptiles. *Journal of Experimental Zoology* **270**: 45–56.
- Viets BE, Tousignant A, Ewert MA, Nelson CE, Crews D.** 1993. Temperature-dependent sex determination in the leopard gecko, *Eublepharis macularius*. *Journal of Experimental Zoology* **265**: 679–683.
- Vogt RC, Bull JJ.** 1982. Temperature controlled sex-determination in turtles: ecological and behavioral aspects. *Herpetologica* **38**: 156–164.
- Zar JH.** 1984. *Biostatistical Analysis*, Second Edition. Englewood Cliffs, NJ: Prentice-Hall.