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Incubation Temperature and Sex Ratio of the Veiled Chameleon (*Chamaeleo calypttratus*)

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ABSTRACT.—Eggs from five clutches of *Chamaeleo calypttratus* were incubated at 25, 28, and 30°C during the period of sex determination. Sex ratios were slightly biased toward females at all temperatures but did not differ statistically from the expected 1:1 ratio of males and females. Egg survival was sufficiently high that sex-biased temperature-induced mortality cannot account for the lack of departure from 1:1 sex ratios. I conclude that the veiled chameleon has genetic sex determination (GSD) and that anecdotal accounts of temperature-dependent sex determination (TSD) for this species, and other chameleons are likely to reflect reporting or statistical bias.

For crocodylians, tuatara, some turtles, and some lizards, temperatures experienced by embryos during incubation affect their sex (Janzen and Paukstis, 1991; Valenzuela and Lance, 2004). For lizards, temperature-dependent sex determination (TSD) is well documented for some species of agamids, scincids and gekkotans (Harlow, 2004). TSD may occur in other families of lizards as well, but reports to date are anecdotal (Harlow, 2004). The problem is that temperature-related sex-ratio bias occurs for reasons other than TSD (Valenzuela et al., 2003). For example, temperature-induced sex-biased mortality during incubation could incorrectly give the appearance of TSD. Reports of TSD could also be statistical artifacts: by chance alone a small

number of temperature-biased sex ratios will occur. These apparent sex ratio anomalies (for a GSD species) would be more likely to be noticed and reported than sex ratios not differing from the expected 1:1 ratio of females and males.

The family Chamaeleonidae is one of the taxa for which TSD has been suggested on the basis of anecdotal accounts (Harlow, 2004). The objective of this paper is to report results of incubation experiments on the veiled chameleon *Chamaeleo calypttratus*. Although the study was conducted for purposes other than assessing TSD (unpubl.), results pertinent to TSD are reported here because they (1) document convincingly that incubation temperature does not affect sex

and (2) support a null hypothesis that is likely to be correct for *C. calypttratus* and perhaps the family as well.

MATERIALS AND METHODS

Eggs were obtained from a breeding colony in my laboratory. Clutch size in captivity is typically 30–40 eggs or more but likely less in nature (Necas, 1999). The five clutches used for this study were laid between 3 and 25 April 2004. Eggs from each clutch were weighed and numbered sequentially with a fine India ink pen within 24 h of oviposition and placed in a plastic “shoe box” container. Eggs were incubated in moistened ecopeat (High Sierra Exotics, P. O. Box 1996, Quincy, CA 95971) at a water potential of -280kPa (150 g H_2O per 100 g ecopeat) based on a standard curve established by vapor pressure psychrometry that related water content to water potential (unpubl. data). Eggs were buried leaving roughly one-third of their top surface exposed so that they could be monitored without disturbance. Water was added to the containers once or twice a week to return the water content to 150%. Eggs were incubated at 25, 28, or 30°C in three Percival incubators. Temperature within an egg container in each of the three incubators was checked daily; temperatures were adjusted if necessary to maintain the targeted temperature. Means recorded within the egg containers during the incubation period averaged 24.8, 28.0, and 29.9°C. Containers were rotated within each chamber several times a week to minimize the effect on development of temperature gradients within the chamber.

The general objectives of the experiment were to assess the effect of incubation temperature on the phenotypes of hatchlings and to determine whether the time of exposure to relatively low and relatively high temperature would modify these effects. Embryos of *C. calypttratus* are gastrulae at oviposition and their development arrested (in diapause) for 60–80 days following oviposition (Andrews and Donoghue, 2004). Eggs were thus incubated at 28°C for 70–72 days at which time a single egg from each clutch was opened and its stage checked using criteria of Dufaure and Hubert (1961). All embryos had initiated development (Stages 11–23) and experimental temperatures were thus imposed at this time (Table 1). One egg from the 28°C treatment from each clutch was also checked at 100 days to determine whether embryos had reached stage 35. Sex is fixed by this stage and, thus, is not influenced by any further change to incubation temperature. Embryos from three of the clutches were at stage 35 at 100 days; thus, some eggs were switched to experimental treatments (Table 1). Embryos from two of the clutches were at Stage 33.5, and these were switched after 3–4 more days based on developmental rates observed in previous studies (Andrews and Donoghue, 2004; Andrews 2004). Embryos that were staged were preserved for other studies.

Incubation temperatures were selected that were known to result in high survival of eggs of *C. calypttratus* in captivity (Necas, 1999; Schmidt, 2001). These observations indicated that 25, 28, and 30°C would represent relatively low, moderate, and high incubation temperatures, respectively. This temperature range also matches the range of mean nest temperatures of *Chamaeleo chamaeleon* during its summer incubation period in nature (C. Díaz-Paniagua, pers. comm.). For

TABLE 1. Incubation temperatures experienced by eggs of *Chamaeleo calypttratus* after incubation at 28°C for 70–72 days. Treatments are indicated as, for example, 28/25 for eggs incubated at 28°C through Stage 35 and at 25°C thereafter. Eggs from each clutch were randomly allocated to the five temperature treatments. Incubation temperature during the sex determining period (Stages 11–35) is the relevant temperature for assessing the effect of temperature on sex ratio.

Treatment designation	Temperature during Stages 11–35	Temperature during Stages 36 to hatching
25/25	25	25
28/25	28	25
28/28	28	28
28/30	28	30
30/30	30	30

lizards with TSD, a range of 25–30°C is sufficiently wide to produce female dominated and male dominated sex ratios (Harlow, 2004).

Eggs from each clutch were randomly allocated to five treatments once development resumed (day 70–72): (1) eggs continued to be incubated at 28°C with no change in temperature during incubation (28/28); (2) change to 25°C for the remainder of incubation (25/25); (3) change to 30°C for the remainder of incubation (30/30); (4) no change in temperature until embryos reached stage 35, then a change to 25°C (28/25); (5) no change in temperature until embryos reached stage 35, then a change to 30°C (28/30; Table 1).

Sex determination in reptiles is associated with gonadogenesis (Bull, 1987). For the lizard *Sceloporus undulatus*, gonads are undifferentiated at Stage 30 but distinguishable as ovaries or testes by stage 35 (Austin, 1988). Only the incubation temperatures that embryos experienced from 70–100 days (stages 11–35) are, thus, assumed to be relevant for assessing sex ratio. Analyses thus contrast the combined 28/25, 28/28, and 28/30 treatments (incubation at 28°C during the sex determining period) with the 25/25 and 30/30 treatments (incubation at 25 and 30°C, respectively, during the sex determining period), although for heuristic purposes observations from all five treatments are reported.

Containers were checked daily for hatchlings from 25 September when the first egg hatched to 3 December when the last egg hatched. At hatching, lizards were identified individually with numbers written on their sides with a “Sharpie” marker and weighed. They were sexed on the basis of a sexually dimorphic character; males have a conspicuous heel spur, females do not. The association between the presence or absence of the heel spur and sex was confirmed by dissecting 13 individuals at about one month of age; in all cases gonadal sex corresponded to the presence or absence of a heel spur. Moreover, all individuals remained the sex initially assigned as they grew.

RESULTS AND DISCUSSION

Results reported here do not support the suggestion that sex ratios of *C. calypttratus* are affected by incubation temperature (Schmidt, 2001). Overall, 98 of

TABLE 2. Percent of eggs surviving incubation and sex ratio at three incubation temperatures for five clutches of *Chamaeleo calypttratus*. See Table 1 for temperature treatment designations. Deaths represent the number of eggs that died prior to allocation to the experimental treatments and Sampled represents the number of eggs used to determine embryo stage. Two hatchlings from the D4 clutch and one from the T6 clutch escaped and thus were not sexed.

	Clutch (clutch size)					% Surviving
	E7 (38)	D4 (57)	T6 (35)	S3 (50)	C5 (54)	
Deaths, sampled	0, 3	2, 3	2, 2	1, 5	9, 6	
Hatched, not hatched						
25/25	12/0	19, 0	10, 0	14, 1	6, 0	98.4
28/25	4, 0	5, 0	3, 0	4, 0	9, 1	96.1
28/28	4, 0	5, 0	4, 0	4, 2	5, 0	91.7
28/30	2, 2	4, 1	3, 0	4, 0	7, 1	83.3
30/30	7, 4	16, 2	10, 1	16, 0	8, 2	86.4
%Surviving	82.9	94.2	96.8	93.3	89.7	
Females, Males						% Female
25/25	6, 6	12, 5	5, 4	3, 11	4, 2	51.7
28/25	3, 1	2, 3	1, 2	2, 2	2, 7	40.0
28/28	2, 2	3, 2	2, 2	4, 0	3, 2	63.6
28/30	1, 1	2, 2	2, 1	3, 1	4, 3	60.0
30/30	3, 4	11, 5	6, 4	7, 9	5, 3	56.1
% Female	51.7	54.0	55.2	45.2	51.4	

the 182 hatchlings in this study were females, and 84 were males (Table 2). Although these numbers reflect a slight female bias, they did not differ from the expected equal numbers of females and males ($\chi^2 = 2.9$, $P > 0.05$). Sex ratios favored females at all incubation temperatures as well with 51.7, 53.7, and 56.1% females from the 25, 28, and 30°C treatments, respectively, and did not differ among treatments ($\chi^2 = 1.0$, $P > 0.05$).

High egg survival, 92% overall, indicates that any sex ratio bias due to differential mortality would be negligible. For example, the slight overall female bias at all temperatures could be explained by higher mortality of male embryos. However, assuming all mortality to have been incurred by female embryos at 25°C ($N = 1$) and all mortality to have been incurred by male embryos at 28°C ($N = 7$; given that TSD produces males at 25°C and females at 28°C as illustrated in figure 5.1 in Harlow, 2004), resultant sex ratios would not differ from 1:1 (25°C; 30 females, 28 males; 28°C: 36 females, 31 males, $\chi^2 = 0.8$, $P > 0.05$). Finally, inspection of the sex ratios from the five clutches does not indicate any clutch-specific bias in sex ratio that could obscure TSD.

Anecdotal suggestions of temperature-related sex ratio bias in *Furcifer lateralis*, *Chamaeleo chamaeleon*, and *C. calypttratus* (Schmidt et al., 1994a,b; Necas, 1999) likely reflect reporting or statistical bias rather than TSD. Results of this study and the observation that sex

ratios of *Furcifer pardalis* hatchlings did not differ from 1:1 when eggs were incubated at either 23 or 28°C (pers. comm. from G. Ferguson in Viets et al., 1994) support the conclusion that sex of chameleons is genetically determined. The Chamaeleonidae includes a large number of species and represents several major adaptive radiations (Townsend and Larson, 2001; Raxworthy et al., 2002), however, and carefully controlled tests for TSD could be made systematically for species in different lineages to determine if TSD actually occurs in the family.

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