Patterns of Temperature-Dependent Sex Determination in Turtles

MICHAEL A. EWERT, DALE R. JACKSON, AND CRAIG E. NELSON
Department of Biology, Indiana University, Bloomington, Indiana 47405-6801 (M.A.E., C.E.N.); and Florida Natural Areas Inventory, Tallahassee, Florida 32303 (D.R.J.)

ABSTRACT   Among reptiles that show temperature-dependent sex determination, sex ratios vary across constant incubation temperatures in ways sufficiently predictable to allow classification into patterns. One common pattern shows low temperatures yielding only males and high temperatures yielding only females. Another common pattern has low as well as high temperatures yielding only or mostly females and some intermediate temperatures yielding mostly males. Patterns tend to be associated with the direction of sexual dimorphism in adult size, especially for species with strong dimorphism.

Pivotal temperatures (those yielding 1:1 sex ratios) within the best-documented species and genera tend to increase with both latitude and longitude across central and southern North America. These geographic trends probably reflect factors that affect nest temperatures (duration of growing season, insolation, and prevailing amounts of shading by vegetation).

Data from a population of the alligator snapping turtle *(Macroclemys temminckii)* suggest that some embryos are temperature-independent females because these individuals become females even when they are shifted among male-producing temperatures during development. These individuals are also more frequent in clutches of small eggs. In this and several other species, no constant incubation temperatures yield more than 75% males.   © 1994 Wiley-Liss, Inc.

As is now widely known, many reptiles have temperature-dependent sex determination (TSD), a form of environmental sex determination (ESD). The Charnov-Bull ('77) model offers an explanation for the adaptive nature of various forms of ESD, including TSD. This model is attractive on theoretical grounds and seems applicable to some examples of ESD in invertebrates that otherwise would be quite puzzling (Bull, '83). Most pertinently, it explains one vertebrate case, a species of fish, in which TSD and genetic sex determination are variously admixed along a geographic gradient (Lagomarsino and Conover, '93). Despite these successes, we lack clear evidence that this model can easily explain the adaptive functions of TSD in reptiles (Bull and Charnov, '89; Ewert and Nelson, '91; Burke, '93).

For the Charnov-Bull model to apply to reptilian TSD (in which sex is determined during incubation), one or more embryonic thermal regimes would have to affect the relative fitnesses differentially in the two sexes. This is not evident in reptilian species that lack TSD except in one report of sexually biased mortality at very extreme temperatures (Burger and Zappalorti, '88). The attempts to show that sexually biased fitness re-sults from various embryonic thermal regimes in TSD reptiles have focused on post-hatching growth, under an assumption that this growth is correlated with lifetime reproductive fitness. These studies on growth have yielded inconsistent results and have often failed to allow the post-hatching young their thermal choice, which is an important consideration (Lang, '87). Nevertheless, a few studies have shown statistically significant differences in growth among cohorts of hatchlings subjected to different incubation regimes (Joanen et al., '87; Brooks et al., '91; McKnight and Gutzke, '93). These results suggest that more careful and consistent experiments involving growth may yield some support for the applicability of the Charnov-Bull model to TSD in reptiles.

Pursuit of a simple solution to the current lack of support among reptiles for the Charnov-Bull model has been complicated also by the considerable heterogeneity of response to temperature among reptiles that have TSD (Ewert and Nelson, '91). For example, any incubation temperature that produces predominantly males in one TSD species will also produce predominantly females in some other TSD species. (However, incubations at 34°C or warmer thus far are known to produce

mostly or only females.) The failure to demonstrate easily the existence of sexually differential effects of temperature has made the existence of TSD in reptiles seem "enigmatic" (Bulmer, '86) and has led to further examination of alternative hypotheses.

Perhaps the strongest current alternative to the Charnov-Bull model is the possibility that TSD is adaptive not because it might match sex with the embryonic thermal environment (and the physiological consequences thereof), but rather because it might produce adaptively skewed sex ratios (e.g., Ewert and Nelson, '91). This idea finds some support in the relative abundance of female biases in hatchling sex ratios in wild populations (Bull and Charnov, '89; Ewert and Nelson, '91). Further, in some species, females predominate under a variety of incubation regimes, whereas the thermal conditions that produce males are extremely limited (e.g., Webb et al., '92). This might suggest an ingrained bias toward the production of females.

It has seemed unlikely that reptilian species with TSD have the population structure thought to be required for adaptively skewed sex ratios (Ewert and Nelson, '91; Burke, '93). This conclusion may need to be reconsidered in light of a recent analysis suggesting that appropriate conditions do exist in a non-TSD snake that produces female-biased broods (Madsen and Shine, '92). The key factors thought to lead to female biases in this snake are its existence in relatively isolated populations and in numbers low enough that males from the same mother sometimes compete for mates. Similar population structures may not be unusual among TSD reptiles.

An additional line of analysis suggests that the tendency toward female-biased ratios in TSD reptiles may not be a sampling artifact. In some partially TSD fish populations that normally conform well to the predictions of Fisherian selection and the Charnov-Bull hypothesis, temperature-induced sex ratio perturbations that lead to female-biased sex ratios readjust to parity through changes in TSD, whereas perturbations that lead to strongly male-biased sex ratios readjust through enhanced temperature independence (Conover et al., '92). This suggests that TSD systems may have evolved so as to produce and tolerate female biases more readily than male biases.

In this paper we present new analyses that further support an earlier suggestion (Ewert and Nelson, '91) that several forces shape TSD in reptiles. In examining the possibility of an associa-

tion of sex determining pattern with direction of sexual dimorphism in size, we find substantial support for such a suggestion in species with pronounced dimorphism. If valid, this correlation would seem to be best explained by factors like those encapsulated in the Charnov-Bull model. But we also find geographic trends in pivotal temperatures that suggest modifications to avoid extreme biases in sex ratios. These trends would be compatible with Fisherian selection on sex ratios acting so as to move away from a strict allocation of one sex to the specific thermal environments from which that sex would otherwise gain the most in terms of physiology and fitness. Finally, a clutch by clutch analysis for one TSD species suggests that some eggs produce females independently of temperature. This again raises the possibility that at least some TSD systems are, contrary to Fisherian selection, predisposed to producing female-biased sex ratios.

TSD PATTERNS

In temperature-dependent sex determination (TSD), hatchling sex ratios vary among thermal incubation regimes, often from all male to all female. TSD patterns describe the plots of sex ratio against an array of constant incubation temperatures (e.g., Fig. 1). Pivotal incubation temperatures are those in which the male and female determining factors are just balanced (Mrosovsky and Pieau, '91); they yield the 1:1 sex ratios that are evolutionarily stable in large, randomly breeding populations (Fisher, '30; Charnov, '82).
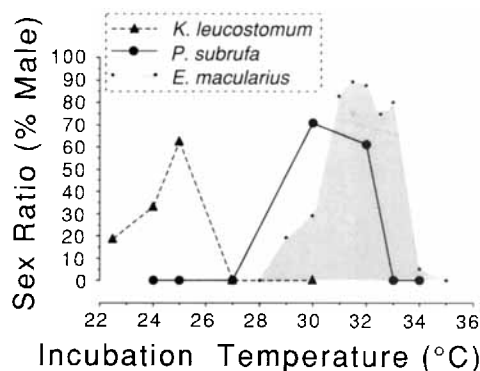

Fig. 1.   Pattern II (FMF) TSD in three reptiles. Examples are selected to contrast relative cool male-producing temperatures in one turtle (Kinosternon leucostomum) with relative warm male-producing temperatures in another turtle (Pelomedusa subrufa) and a lizard (Eublepharis macularius). Data are from Viets et al. ('93) and Ewert and Nelson ('91), except for larger samples K. leucostomum (at 25°C, n = 8) and P. subrufa (at 27°C, n = 18; at 32°C, n = 18; at 34°C, n = 11).

Sex ratios of 1:1 also can be achieved in populations by an appropriate mix of male-biased and female-biased ratios produced under various thermal regimes.

Among the three generally recognized TSD patterns, two have a single pivotal temperature (low temperature females, high temperature males = FM; low temperature males, high temperature females = MF), and one has two pivotal temperatures (low temperature females, medium temperature males, high temperature females = FMF) (Fig. 1). These patterns have been listed, respectively, as Patterns (or Types) A, B, and C or 1, 2, and 3 (Bull, '80, '83; Bull and Charnov, '88), or, with the sequence of the first two patterns reversed, I, II, and III (Bull and Charnov, '89; Deeming and Ferguson, '91). Patterns showing FMF occur in all groups of reptiles known to have TSD (turtles, crocodilians, and lizards). Thus, FMF may be basal or ancestral, and the patterns showing FM (absent in turtles) and MF (expressed only in turtles) may be derived through loss of suppression of females at high or low temperatures, respectively (Deeming and Ferguson, '88). Ewert and Nelson ('91) adopted this hypothesis in designating the basal pattern (FMF) as Pattern II, and the two derived patterns as Pattern Ia (MF) and Pattern Ib (FM). Some workers may prefer to refer to the patterns as well as their features (e.g., pivotal temperatures, transitional ranges in temperature from giving mostly one sex to mostly the other) by the sequence of sexes (e.g., FMF). However, we feel that this procedure can confuse the reader when all three entities appear in complex discussions.

Pattern Ib has been classically assumed to occur in two lizards and some alligatorines (Bull, '80; Gutzke and Crews, '88; Deeming and Ferguson, '89). However, following the demonstration that females predominate at increasingly warmer incubation temperatures, putative Pattern Ib is now redefined as Pattern II, both in the lizard *Eublepharis macularius* (Viets et al., '93, this issue) and in the alligatorines (Lang and Andrews, this issue). The one remaining putative Ib species, the lizard *Agama agama*, remains unstudied since its premier role in associating reptilian sex ratios with incubation temperature (Charnier, '66). Studies at warmer incubation temperatures may well yield predominantly females in this species, too. This leaves us with no well-established examples of Pattern Ib (i.e., no examples for which the warmest viable temperatures have been shown to yield predominantly or exclusively males). This pattern

is clearly even rarer than it once seemed and may even not exist. If so, it will be simpler to refer to Pattern Ia (MF) simply as Pattern I.

Note that in the absence of any clear examples of Pattern Ib species, all well-studied TSD reptiles yield only or mostly females at the warmest constant incubation temperatures at which they are viable. It is predominantly in the responses to cool incubation temperatures (within the array of viable incubation temperatures for each species) that the major difference occurs. Pattern Ia species produce exclusively or nearly exclusively males at these cooler temperatures, whereas Pattern II species produce only or mostly females at the cooler temperatures.

The existence of Pattern Ia (MF) has been solidly established as different from Pattern II (FMF) by showing only males, rather than some females, following the use of very low temperatures (during the thermosensitive period of development, when temperature determines sex) that are distinctly lower than those that will sustain development to hatching (i.e., at 18–19°C [Pieau, '78]). However, use of constant incubation temperatures near the lower limit for sustained development (e.g., at 21.5–22.5°C for many turtles [Ewert and Nelson, '91]) usually distinguishes the two patterns. Further, Pattern Ia usually differs from Pattern II in having a wider range of sustaining temperatures that yield strongly male-biased sex ratios and in reaching fixation (100% male) at some temperatures (often over 2°C in range). In Pattern II species, male fixation is rare (but occurs in some northern *Chelydra* [Yntema, '76; see also Lang and Andrews, this issue]), and sex ratios seldom exceed 80% males at any constant temperature tested (Ewert and Nelson, '91). Pattern Ia, despite its occurrence only in turtles, has nearly worldwide representation largely through its expression in sea turtles and in some of the emydid pond turtles (e.g., *Chrysemys, Emys, Trachemys*).

Pattern II (FMF) species by definition produce predominantly or exclusively females at both relatively cool and relatively warm incubation temperatures and predominantly (though seldom exclusively) males at some intermediate temperatures. However, considerable variation exists among Pattern II species in the range of temperatures at which males predominate. For example, male-producing constant temperatures of the turtles *Pelomedusa subrufa* and *Kinosternon leucostomum* do not overlap, whereas the male-producing temperatures of *P. subrufa* and the lizard *Eublepharis macularius* share appreciable

overlap (Fig. 1). From a functional perspective, embryos of *K. leucostomum* in natural nests would appear to experience their thermosensitive period (TSP) in the MF transitional range. This turtle inhabits neotropical forest ponds and swamps and probably nests nearby, where temperatures would seldom get as cool as the FM transitional range. Although trends in natural nest temperatures of *P. subrufa* and *E. macularius* during their TSPs are not known, incubation at 28°C (in or approaching the FM transitional range) produces vigorous females in both species (Ewert, unpublished; Viets et al., unpublished). Thus, sex determination in or close to the FM transitional ranges could predominate in both of these species, making them functionally Pattern Ib. More generally, however, many, perhaps most, Pattern II turtles have MF transitional ranges that are cool or at least no warmer than those of Pattern Ia turtles. In these Pattern II turtles, sex determination probably predominates in or close to their FM transitional ranges (e.g., *Chelydra serpentina* [Wilhoft et al., '83]). In contrast, the natural incubation temperatures in nests of many Pattern II species of crocodilians and lizards may occur below and across their FM transitional ranges (Ferguson and Joanen, '82; Lang et al., '89), making these species functionally Pattern Ib (FM). However, the MF transitional range of the Pattern II lizard *Gekko japonicus* is cool for a lizard (Tokunaga, '85) but quite like that of many turtles. Hence, sex determination of this lizard probably predominates in or close to its FM transitional range. In summary, there are large differences in TSD patterns both among species and among higher taxa, and these differences reflect predominant expression of transitional ranges (FM or MF) during development in natural nests. However, there are also cases that suggest similarities among higher taxa that are likely to be functionally similar in nature.

Although Patterns Ia and II are often clearly differentiated, it is important to note that not all TSD responses in turtles fit this dichotomy (Ewert and Nelson, '91). Indeed, turtles of the Kinosterninae manifest several intermediate patterns (Etchberger, '91), including one that suggests a blend of TSD and temperature independence (*K. baurii* [Ewert et al., '90]). Kinosternids also include very strong examples of Pattern Ia (*K. hirtipes*) (Table 1) and Pattern II (*Sternotherus minor*) (Fig. 2) (see also Etchberger, '91). This extreme diversity among patterns within a small taxon suggests that the primary dichotomy in patterns (Ia

vs. II) may persist despite an appreciable potential for evolutionary change.

SEXUAL SIZE DIMORPHISM HYPOTHESIS

This hypothesis proposes that TSD pattern and growth potential are related, as if incubation temperature were matching sex with sets of "instructions" for future growth and maturation that would ultimately affect reproductive fitness (Webb et al., '87). Sexual development during incubation would proceed according to the Charnov-Bull hypothesis, with sex determination matching each sex with the appropriate thermal patch (Charnov and Bull, '77). However, observable expression of fitness might be delayed until near maturity. Thus, TSD pattern might be associated with relative size differences between the sexes (Head et al., '87; Webb et al., '87). Ewert and Nelson ('91) find support for the sexual dimorphism hypothesis both within turtles alone and as extended to include crocodilians and lizards. In species with Pattern Ia (MF), females are usually the larger sex, as if warm temperatures fostered growth and delayed maturity and cool temperatures fostered early maturity regardless of achieved growth. In Pattern II (FMF) species, females are usually not larger than males; in Pattern II, males predominate at intermediate temperatures. These temperatures may optimize future growth and maturation, whereas more extreme temperatures (either low or high) may be just adequate for growth and maturation of smaller females (Webb et al., '87). However, these correlations also mean that the sex that is smaller as adults is usually produced at cooler temperatures according to either pattern. Possibly, sex determination at cool temperatures does not produce the fittest individuals of either sex but rather matches the sex that is fitter as relatively small adults to cool conditions (Webb et al., '87; Ewert and Nelson, '91).

Janzen and Paukstis ('91) cluster species into units of similar phylogeny and TSD pattern and find a lack of significant association in the small resultant sample. They do not state how they decided which species had which patterns, but they appear to assign patterns to some species for which data are not sufficient to determine the pattern unambiguously. Also, the direction of sexual dimorphism, as they state it, reveals a few inconsistencies with published data (e.g., for *Pelomedusa subrufa, Testudo graeca,* and *Eublepharis macularius,* all with reverse to stated sexual dimorphism, and for *Terrapene ornata,* with males < females [references in

TABLE 1. Sex ratios as a function of incubation temperature in hitherto unreported species of turtles[1]

Species	Constant incubation temperature (°C)						
	22.5	25	27	28	30	31	32
Kinsternon hirtipes	100 (6)	100(7)	100 (6)		17 (9)[2]		
Heosemys grandis		0 (11)		77 (13)	0 (10)		
Clemmys marmorata		100 (9)	100 (8)		42 (6)[2]	0 (6)	
Graptemys versa		100 (5)			86 (7)		0 (8)

[1]Generally, incubation procedures follow Ewert and Nelson ('91). Throughout the presumed thermosensitive periods for sex determination in the embryos, the incubators functioned within ± 0.3°C of design temperatures (as measured in 400 ml reservoirs of water), with rare deviations of ± 0.5°C. With exception of single incubations of clemmys marmorata at 30°C and 31°C, all incubations were replicated sequentially in the same or different incubators. Results are reported as percent male, followed by sample sizes in parentheses. The data representing each of the four species differ from temperature independence by P < 0.005 as examined by Chi-square tests of independence. Natural origins of samples: K. hirtipes, northern Chihuahua; H. grandis, unknown (captive bred zoo stock); C. marmorata, southern Oregon and northern California; G. versa, Texas (captive bred zoo stock).
[2]One individual was diagnosed as intersexual and assigned a sex of one-half male.

Ewert and Nelson, '91]). Perhaps in both studies (Janzen and Paukstis, '91; Ewert and Nelson, '91), small sample size, presence of at least a few non-supportive species, and lack of strong sexual size dimorphism in many cases argue for deferring a general conclusion.

However, if we focus only on TSD species with strong sexual size dimorphism, the trend favors the sexual dimorphism hypothesis, especially in phylogenetically related pairs of turtles: Pelomedusa subrufa (M > F, Pattern II) vs. Podocnemis expansa and P. unifilis (M < F, Pattern Ia) (Sousa et al., '90; Ewert and Nelson, '91; Lance et al., '92; Ewert and Goode, unpublished); Heosemys

Fig. 2. Comparison of TSD patterns among four species of turtles of the Lower Apalachicola River floodplain, Franklin and Gulf counties, Florida. The two Pattern IIs (FMFs) (M. temminckii, S. minor) share many similarities, whereas the two probable Pattern Ias (MFs) (G. barbouri, T. scripta) also share similarities but differ markedly from the Pattern IIs. Sources and unpublished data (listed as temperature, n = hatchlings, n = nests): M. temminckii: see Fig. 7; S. minor: Ewert and Nelson, '91; Ewert, Etchberger, and Nelson, unpublished; G. barbouri: Ewert and Nelson, '91; 28°C, 12, 6; 29°C, 13, 6; T. scripta: 28°C, 37, 16; 29°C, 19, 8; 30°C, 14, 7.

grandis (M > F, Pattern II) vs. Rhinoclemmys pulcherrima (M < F, Pattern Ia) (Table 1) (Ewert and Nelson, '91); Terrapene carolina (including T. c. major) (M > F modestly, but greatest for any Nearctic emydine turtle, toward Pattern II) vs. Graptemys geographica (M << F, Pattern Ia) (Ewert and Nelson, '91; Ewert and Jackson, unpublished); Kinosternon flavescens arizonense (M > F, Pattern II) vs. K. baurii (M < F, weak Pattern II) (Fig. 2) (Ewert et al., '90, unpublished; Ewert and Nelson, '91; Etchberger, '91). Also, lizards and crocodilians (M > F, Pattern II, one questionable Pattern Ib) remain supportive as two phylogenetically independent groups (Ewert and Nelson, '91; see Lang and Andrews, '94; Viets et al., this issue).

More evidence should be forthcoming as several contrasts remain to be explored among turtles with appreciable sexual size dimorphism and a strong likelihood of TSD. Examples include the batagurine batagurids (e.g., Chinemys, Hardella, Kachuga—all M << F) vs. select geoemydine batagurids (M > F), very large testudinids (M > F) vs. very small ones (M < F), and possibly some species of the African pelomedusid genus Pelusios (possibly M << F) vs. Pelomedusa (M > F) as well as the neotropical genera Peltocephalus (M = F) vs. Podocnemis (all M < F).

What appears to happen to TSD pattern in species with weak sexual dimorphism in size? Patterns in some of these species may become intermediate between II and Ia, as in the Kinosterninae mentioned above (see also Etchberger, '91). In this group the direction of sexual dimorphism in size is variable, both within and among species, and often it is only slight (Iverson, '91). There is variation TSD pattern that is not clearly associated with direction of dimorphism. For example, two subspecies of K.
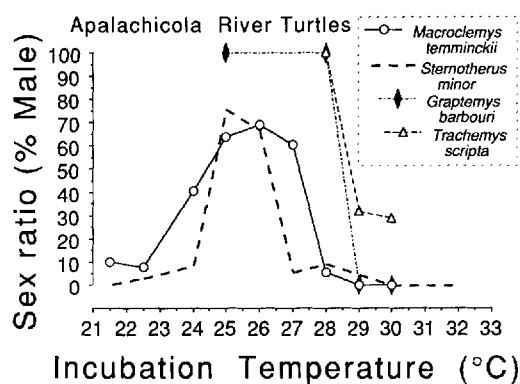
flavescens show significant variation both in the MF pivotal temperatures and in low temperature sex ratios (Fig. 3). In both subspecies, males are larger than females, but this difference is a little smaller (both absolutely and relatively) in *K. f. flavescens,* the more northern subspecies (Iverson, '91). The more northern subspecies has an intermediate TSD pattern despite the reduction in sexual dimorphism being relatively slight (although critically needed data at 28 or 29°C could increase the resemblance to Pattern II). The strong contrast in patterns suggests that factors other than just sexual size dimorphism do contribute to the evolution of TSD pattern, at least when the extent of dimorphism is relatively small.

Our purpose here is not to argue that the sexual size dimorphism hypothesis has been proven conclusively but rather to caution against its premature dismissal. The present trend is toward significant association in related groups when dimorphism is appreciable. This may eventually reveal something about the adaptive value of TSD even if sexual size dimorphism per se is not a major factor in selection for TSD.

GEOGRAPHIC VARIATION IN PIVOTAL TEMPERATURES

The variation in pivotal temperatures (those that give 1:1 sex ratios) across species and major taxa is much greater than the geographic variation within any species. Across species, MF pivotal temperatures of Pattern Ia (or probable Ia) species vary from approximately 27.5°C in *Chrysemys picta* (Schwarzkopf and Brooks, '85) to >32.5 to <34°C in *Podocnemis expansa* (Alho et al., '85; Lance et al., '92). The range of MF pivotal temperatures in Pattern II (FMF) species is from 25.5°C in *Kinosternon leucostomum* (Fig. 1) or 25.6°C in Florida *Chelydra serpentina* (Fig. 4) to ~32.1°C in *Pelomedusa subrufa* (Fig. 1). Pattern II FM pivotal temperatures range from <22°C in northern *C. serpentina* (Yntema, '76; J.W. Lang, unpublished) to ~29°C in *P. subrufa* (Fig. 1), with most species having FM pivotal temperatures well below 27°C.

Pivotal temperatures of sea turtle rookeries are conserved within a ~1°C range (28.6–29.7°C) and lack clear geographical trends (Mrosovsky, this issue). However, geographic trends, while seemingly slight, do occur in freshwater species. The best-established trend shows an intraspecific increase in pivotal temperatures with increase in latitude (Fig. 4) (Bull et al., '82b; Vogt and Flores-Villela, '92). However, a longitudinal trend also is evident (Fig. 5), such that the main vector for increase in pivotal temperatures of *Chrysemys picta* across mid-America appears to be southeast to northwest. Interspecific variation in *Graptemys* also supports a longitudinal trend (Fig. 6). The overall trend in North America appears to distinguish species and populations of forested, humid, often cloudy eastern climates from those of sunnier, prairie or semidesert, western climates.
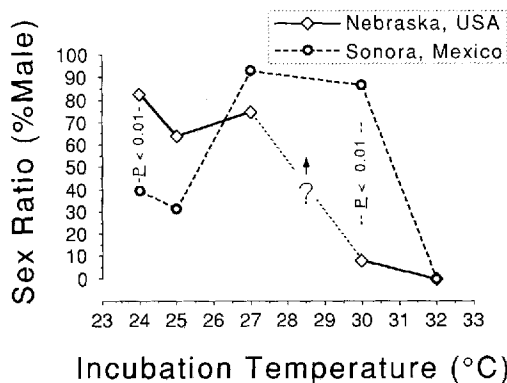

Fig. 3. Geographic variation in TSD pattern in *Kinosternon flavescens*. Incubation methods and diagnoses of sex follow Ewert and Nelson ('91). Tests of homogeneity of sex ratios at 24°C and 25°C used Chi-square. Patterns for other kinosternids suggest that strongly male-biased sex ratios may well occur at 28°C or 29°C in samples of the Nebraskan population. Sample sizes at given temperatures: for Nebraska (Garden Co.): 24°C, n = 23; 25°C, n = 25; 27°C, n = 20; 30°C, n = 12; 32°C, n = 3; for Sonora, Mexico (most eggs produced in captivity): 24°C, n = 24; 25°C, n = 19; 27°C, n = 14; 30°C, n = 15; 32°C, n = 11.

Fig. 4. The MF pivotal temperatures of two turtle species tend to increase with latitude. The Spearman Rank correlation coefficient for *C. serpentina* is 0.69 ($P < 0.05$). The three samples of *T. scripta* at latitudes 30–31°N express longitudinal variation (compare Figs. 5, 6), as the lowest of these pivotal temperatures is from Florida and the highest is from central Texas. Sources of data for *C. serpentina:* Brooks et al. ('85), Janzen ('92), Schwarzkopf and Brooks ('85), Vogt and Flores-Villela ('92), and Ewert and Lang (unpublished); for *T. scripta:* Etchberger et al. ('92), Lockwood et al. ('91), Vogt and Flores-Villela ('92), and this study (see Fig. 2).

Longitude (°W)

Fig. 5. The MF pivotal temperatures of *Chrysemys picta* plotted against geographic coordinates in central North America. Unpublished pivotal temperatures are estimated by simple linear projection between the two most closely bracketing values. Sources and unpublished data, from west to east: Nebraska: Packard et al., '89; NW Minnesota: Lang and Ewert, unpublished (50% male at 29°C, n = 20); SE Wisconsin: Bull et al., '82b; NW Tennessee: Bull et al., '82b: this study (90% male at 27°C, n = 10); central Indiana: this study (100% male at 27°C, n = 46; 48% male at 28°C, n =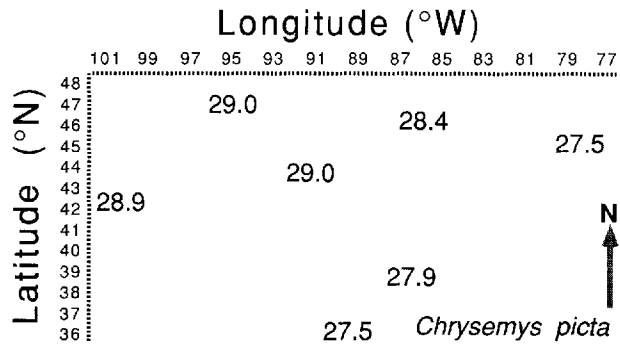 17); northern Michigan: this study (100% male at 27°C, n = 10; 83% male at 28°C, n = 12); eastern Ontario: Schwarzkopf and Brooks, '85.

Indeed, eastern (including southeastern) species or biotypes of freshwater turtles with TSD most often nest in only moderately warm places relative to sites available for nesting in the prairies or arid southwest. Some southeastern species or biotypes clearly choose shade (*Chelydra* in the southeast [Ewert, '76; Ewert, Jackson, and J.W. Lang, unpublished]). Other species or biotypes (*Chrysemys* in western Tennessee, *Trachemys* in
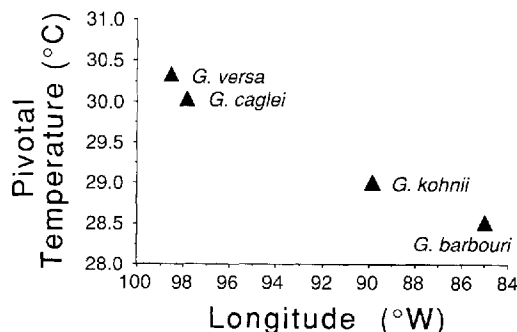

Fig. 6. The MF pivotal temperatures among four riverine *Graptemys* species tend to increase with longitude. All populations occur at approximately 29–31°N latitude, except that of *G. kohnii*, which is from 36°25′N. Sources and unpublished data, from west to east: this study, Table 1; Wibbels et al., '91; Etchberger, '91; this study (see Fig. 3).

Florida, some *Graptemys* and *Pseudemys*) choose or at least accept partial shading or filtered sunlight (Ewert and Jackson, unpublished; see also Vogt and Flores-Villela, '92). In the northeastern US and eastern Canada, extensive forests (prior to European settlement) may have resulted in a scarcity of warm nesting sites. Pivotal temperatures are low to very low and would apparently allow adequate production of females across and above the MF transitional range. More to the northwest (e.g., northern prairie), warm sites are readily available, but only during the brief growing season. Here, all turtles seek warm, open nesting sites that will allow rapid development and hatching before winter. Pivotal temperatures when sufficiently high will provide some males at temperatures that might otherwise produce only females. In the semiarid southwest, pivotal temperatures might have to accommodate a general absence of cool nesting sites for spring- and summer-nesting species, such as most of the ones being considered here. Other turtles that follow this general trend are northeastern *C. guttata* (pivotal temperature <30°C [Ewert and Nelson, '91, unpublished]) vs. northwestern *Clemmys marmorata* (from seasonally arid south-central Oregon; pivotal temperature ~30°C) (Table 1) and all eastern and neotropical forest kinosternines (pivotal temperature <28.5°C [Etchberger, '91; Ewert and Nelson, '91]) vs. *Kinosternon flavescens arizonense* (from low desert in Sonora; pivotal temperature >30°C (Fig. 3). The highest pivotal temperature known among turtles (>32.5 but <34°C in *Podocnemis expansa* [Alho et al., '85; Lance et al., '92]) accommodates a need for rapid embryonic development to avoid drowning in riverine sandbar nests with extremely high temperatures (to 34.5°C mean and 38.5°C maximum attained in this environment [Alho et al., '85]). Thus, the original hypothesis of Bull et al. ('82b), that pivotal temperature can vary and can reflect regional climate, is supported from the perspective that there are constraints for nesting in warm or cool microclimates.

Choice of nesting sites also corresponds to pivotal temperature within a single region, the Apalachicola River floodplain (Fig. 2). Nest sites of *Graptemys barbouri* and *Trachemys scripta* tend to be located toward the edges of man made open sites or in filtered sunlight or light shade. Nests of *Macroclemys temminckii* vary from fully open to fully shaded. However, the eggs are buried to appreciably greater depths than in the other species. *Sternotherus minor* often nest in cool sea-

sons and in partial to full shade (Ewert and Jackson, unpublished). Within the broader geographic context, note that the highest pivotal temperature among Apalachicola populations is 28.7°C, whereas pivotal temperatures of 29–30°C are common toward the northwest and west, across the central US.

LIMITS TO MALE-BIASED SEX RATIOS IN PATTERN II

In most Pattern II species, no single constant incubation temperature, even within large, moderately tightly spaced (1°C) experimental arrays, seems able to produce 100% males. In the most extreme case, *Crocodylus johnstoni* yields sex ratios never greater than 39% at constant temperatures (Webb and Smith, '84; Webb et al., '90, '92). Simple daily alternation of temperatures between 31°C and 33°C does not significantly increase male proportions (Webb et al., '87, '90, '92). However, incubation of modest to large samples of eggs through a select series of small increases in temperature (either as increments of ~1°C widely spaced over several days or as a very gradual rise of <<1°C/day; generally beginning at 30–31°C and ending at 33–35°C) yields sex ratios of 56–88% male. One conclusion is that most of the constant temperatures and combinations of constant temperatures that sustain embryonic development discourage male development in *C. johnstoni*. Another is that even the most optimum known combination of temperatures for male development does not seem to foster male development in every egg. Thus, males are difficult to induce, and some eggs may be incapable of becoming male at any temperature or combination of temperatures.

The alligator snapping turtle (*Macroclemys temminckii*) also fails to yield all males across clutches at any constant temperature (Fig. 7). Perhaps, as in *Crocodylus johnstoni*, a closely defined pattern of shifts in temperature fosters male development. If so, although many combinations of temperature are possible, some increase in the proportion of males as a result of shifting eggs among temperatures would suggest a trend that could be enhanced through refined experiments. A second hypothesis is that the best temperatures for fostering male development might differ slightly among embryos. If so, exposure of embryos to pulses of all temperatures that give male-biased sex ratios should increase the proportion of males. Each embryo would have received at least some exposure to the temperature most influential in making it into a male within an otherwise
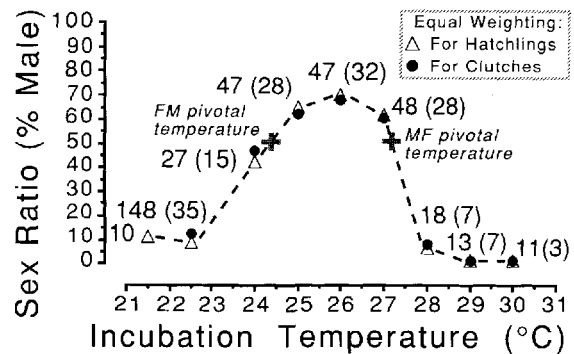

Fig. 7. TSD pattern for *Macroclemys temminckii* at constant incubation temperatures. Sample sizes of diagnosed hatchlings (number of clutches represented) are provided next to each plotted point. Survival through hatching is high at all temperatures except at 21.5°C.

neutral to slightly masculinizing array of temperatures. A third hypothesis is that some embryos form females in a temperature-independent but sex-biased response within a system normally conceptualized as purely TSD. This hypothesis would be supported if the samples from some clutches maintain females at all treatments to which they are subjected, whereas other clutches yield samples that readily produce all or nearly all males.

Our test protocols assumed that *M. temminckii* follows *Chelydra* in having a relatively early and brief TSP (Yntema, '79) but with perhaps a little prolongation as in emydids (Pieau and Dorizzi, '81). One protocol ("single shift" or "single step-shift" protocol) used a single series of two step-shift increases in temperature (Fig. 8A) (designed after Webb et al., '87 but adjusted to *M. temminckii*). If the first hypothesis (i.e., a response as in *C. johnstoni*) were true, this should produce an appreciable increase in the observed proportion of males. In a second approach ("shift rotation" protocol), we rotated two boxes of eggs (box A and box B), each box on a separate 3 day cycle, among incubators set at 25, 26, and 27°C (Fig. 8B)—that is, the three constant temperatures that yield male-biased sex ratios (Fig. 7). In this protocol, all embryos should experience all temperatures between 25°C and 27°C during the TSP regardless of the exact duration of the TSP. If our second hypothesis (heterogeneity in male optima) were true, this should produce an appreciable increase in males. Eggs for the control treatment were incubated at comparable constant temperatures. Because of a protected status and a concern for the conservation of alligator snapping
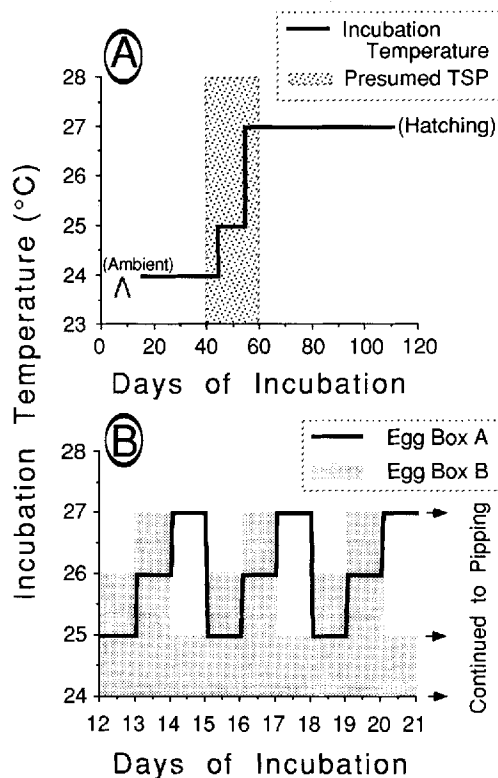
Fig. 8. Two thermal protocols used for incubating eggs of *Macroclemys temminckii*. Under Protocol A, eggs incubating at 24°C were candled approximately daily to assess embryonic stage. The first transfer was made at Yntema ('68) Stages 15–16; the second transfer followed in 10 days. Under Protocol B, eggs were assigned to incubation boxes in a quasi-latin square design in which clutchmates occupied different rows and both internal and side positions. TSP = thermosensitive period; see text for additional explanation. All eggs were collected from natural nests and repeatedly candled to confirm fertility and, later, embryonic stage at the initiation of the protocols. Incubation media consisted initially of a 1:1 mixture of vermiculite and water; later, additional water was added.

Fig. 9. Proportions of sexes in hatchlings of *Macroclemys temminckii* following incubation at single constant temperatures or according to the temperature shift protocols A (a single series of step-shifts) and B (3 day shift rotations) shown in Fig. 8. Treatment sample sizes of diagnosed hatchlings (clutches) are listed above each vertical bar.

turtles, we seldom used more than eight eggs from each clutch (~35 eggs) for this work.

The temperature shift protocols and constant temperature treatments yielded similar sex ratios (Fig. 9). The combined sex ratio from three constant temperatures and the overall sex ratio from the shift rotation protocol that employed the same three temperatures differed by less than 1%, although the two series were derived from different series of clutches and different years (Fig. 9). As we drew eggs from the same clutches to use for incubation at 26°C and for the single step-shift protocol, we examined the comparison with Wilcoxon's signed rank test (two treatments, 25 clutches) and found no significant difference

among treatments. Thus, our results gave no support to either of the first two hypotheses.

In addition to our formal protocols, we took 1–3 eggs from some of the clutches involved in the shift rotation protocol and buried them as a group at a cool, shaded, but otherwise typical site for nesting at our Apalachicola flood plain field site. This nest yielded 65% males (13 males, 7 females), which is close to (and not significantly different from) the sex ratio obtained from the shift rotation protocol. Thus, we lack any evidence that some protocol involving sequential temperatures may increase the proportion of males in *M. temminckii*.

Male-biased sex ratios and intra-clutch variation in Pattern II

Some of the clutches of eggs from *M. temminckii* responded to the three constant temperatures (25, 26, and 27°C) by producing only males, whereas others produced both sexes at these temperatures. Clutches that produced only males at all three constant temperatures were significantly more likely to produce only males under the single step-shift protocol in temperature (Fig. 10). Likewise, if clutch samples in one box (A or B) of the shift rotation protocol (Fig. 8B) produced only males, samples from the same clutches were significantly more likely to produce only males in the other box of the shift rotation protocol (Fig. 10) without these boxes ever having been in the same incubator at the same time. Such resemblances between

Fig. 10. Concordance of sex ratios among clutch sub-samples for *Macroclemys temminckii*. **Left side**: Concordance of one subsample incubated at constant male-biased temperatures (see text) with a second subsample from the same clutch incubated in a single step-shift (protocol A of Fig. 8). **Right side**: Concordance within the shift rotation (protocol B of Fig. 8) of one subsample (Box A) from each clutch with a second subsample (Box B) from the same clutch. Only clutches in which both subsamples could be unambiguously assigned to category (i.e., most or all of the eggs in both subsamples hatched as necessary) are represented in this analysis.

samples from the same clutches are commonly cited as implying substantial heritable variation in sex determination (e.g., Bull et al., '82a; Janzen, '92). Although the heterogeneity among clutches is significant, the ultimate strength of our imposed dichotomy (all males vs. one or more females) is difficult to assess when our samples represented just 4–6 eggs in clutches ranging typically from 20–45 eggs. However, we did expose one entire clutch (40 eggs, 37 hatchlings diagnosed) to the step-shift increase protocol and obtained 36 males and 1 female.

One conclusion from this work is that embryos within certain clutches are relatively less likely to become males. Given that all incubation focused on the male-biased thermal range for *M. temminckii*, the embryos that become males presumably show the conventional sexually bipotential state that is assumed in reptilian TSD. These embryos almost certainly would have become females at 29°C or 30°C, because no embryos become males at these temperatures.

What can be concluded from the various incubation protocols about the embryos that became females despite incubation within the male-biased thermal range? Although we could have a phenomenon in which only a very narrow set of conditions yield males (as found for *C. johnstoni* [Webb et al., '87, '92]), we were unable to find any evidence for

this. Rather, it seems that some embryos become females regardless of their thermal environments during development; that is, they are temperature independent. This would not necessarily require a female-biasing difference in the genotypes of the embryos. Rather, the mothers might differ in the extent to which the contents of the eggs contained a feminizing (i.e., estrogen) endocrine environment (Crews et al., '91; Dorizzi et al., '91).

Functionally, the presence of temperature-independent females without temperature-independent males can exist within Fisherian limitations, given that many natural nests continue to yield predominantly to nearly exclusively males (Ewert and Jackson, unpublished). Temperature-independent females would constitute a clear advantage during prolonged environmental perturbations that result in male-biasing annual recruitments. Still, it may be difficult to accept that nearly one-third of all embryos may be temperature-independent females.

A correlate of intraclutch variation in sex ratio

We have records of mean egg mass (based on 4–6 eggs/clutch) for the samples exposed to the shift rotation protocol as well as of the total number of eggs in the sampled clutches. Clutch-sample sex ratio is significantly correlated with sample mean egg mass (Fig. 11). Clutch-sample sex ratio is also significantly correlated with clutch mass (Spearman $r_s = 0.67$; $P < 0.01$), as estimated from mean sample egg mass × clutch size. Sex ratio is not significantly correlated with clutch size alone. Given that mass typically equates with energy

Fig. 11. Clutch sex ratio vs. clutch-mean egg mass in *Macroclemys temminckii*. The sex ratios are those resulting from the shift rotation protocol (B of Fig. 8) and represent all 18 clutches from this treatment (15 clutches with 4 diagnoses/clutch; 3 clutches with 3 diagnoses/clutch). Mean egg mass for each clutch was computed from measurements on 4–6 recently laid eggs/clutch including all of the eggs that later produced diagnosible late embryos or hatchlings (Spearman $r_s = 0.61$; $P < 0.01$).

content, we believe that our correlations represent the first report of an association between parental investment and allocation of sex within the framework of reptilian TSD. However, the best theoretical framework for assigning adaptive value to our observation remains equivocal. We could have a Trivers-Willard ('73) optimization of sex with egg mass, because males from large eggs might gain relatively more from receiving a small headstart toward ultimately becoming the larger sex. However, studies so far (Etchberger, '91; Brooks et al., '91) have failed to demonstrate any association of egg size (or hatchling size) with early growth potential in turtles.

Alternatively, note that egg size and egg mass are both positively correlated with maternal size in the related species, *Chelydra serpentina* (Yntema, '70), and that old *C. serpentina* tend to be larger than young ones (Galbraith, '86). If *M. temminckii* is similar, our data (Figs. 10, 11) suggest that the heterogeneity in sex ratios could be correlated with female age.

Within a kinship framework of local mate competition, our observations might invite an explanation parallel to that offered for a viperid snake (Madsen and Shine, '92), in which competition among brothers leads to selection on females for female-biased sex ratios. However, in the snakes, the female bias in litters increases with age, whereas in our turtles the female bias would tend to decrease with age, as fewer temperature-independent females are produced. Still, alligator snapping turtles are likely to be moderately sedentary, and older males are likely to compete for mates and, as in *Chelydra* (Kiviat, '80; Galbraith et al., '86), to be partially territorial. Also, investment in female-biased offspring sex ratios early in adult life might have advantages in colonizing vacant habitat (Ewert and Nelson, '91). Nevertheless, we remain puzzled as to why female-biased investment might decrease with maternal age.

In response to our highly selective manner of identifying temperature-independent females against a background of males, it is logical to question relevancy to expression in the natural environment. Natural nest temperatures typical of those yielding males and temperature-independent females may once have predominated in our study area. Nesting habitat, probably mostly along river berms, would once have been mostly shaded with floodplain forest. Currently, however, many nesting females, regardless of their clutch masses (or ages), are nesting in artificially created open areas that have nest temperatures so warm that they nearly always yield female-biased sex ratios (Ewert and Jackson, unpublished), as expected through normal TSD.

CONCLUSIONS

Although the Charnov-Bull hypothesis remains a viable explanation for the adaptive value of TSD, it suffers from lack of a clear association of sexual differential adult fitness with embryonic incubation temperature. The Charnov-Bull hypothesis might be supported by an association of TSD with sexual dimorphism hypothesis, but the latter needs further verification and, especially, a link between incubation temperature and adult fitness. Pivotal temperatures of freshwater turtles do show geographic variation. This variation can be explained with the assumption that selection has acted to maintain similar sex ratios across populations in ways that may modify the associations between given temperatures and sexually differential fitness. However, general seasonal trends in temperatures, as well as random and diel fluctuations in nest temperatures, are also likely to vary geographically and may at least partially dissociate pivotal temperatures from temperature ranges that are most relevant to sexually differential fitness. Lastly, TSD, particularly within Pattern II, shows a bias toward the production of females that invites speculation on adaptive value beyond the Charnov-Bull hypothesis.

LITERATURE CITED

Alho, C.J.R., T.M.S. Danni, and L.F.M. Padua (1985) Tempera-ture-dependent sex determination in *Podocnemis expansa* (Testudinata: Pelomedusidae). Biotropica, *17*:75–78.

Brooks, R.J., E.G. Nancekivell, and D.A. Galbraith (1985) Sex determination and hatchling sex ratio in northern population of *Chelydra serpentina*. Proc. Ann. Meet. SSAR and HL, Univ. So. Fla., Tampa, FL, 4–9 August 1985, *28/33*:40.

Brooks, R.J., M.L. Bobyn, D.A. Galbraith, J.A. Layfield, and E.G. Nancekivell (1991) Maternal and environmental in-fluences on growth and survival of embryonic and hatchling snapping turtles (*Chelydra serpentina*). Can. J. Zool., *69*:2667–2676.

Bull, J.J. (1980) Sex determination in reptiles. Q. Rev. Biol., *55*:3–20.

Bull, J.J. (1983) Evolution of Sex Determining Mechanisms. Benjamin/Cummings, Menlo Park, CA.

Bull, J.J., and E.L. Charnov (1988) How fundamental are Fisherian sex ratios? Oxford Surv. Evol. Biol., *5*:96–135.

Bull, J.J., and E.L. Charnov (1989) Enigmatic reptilian sex ratios. Evolution, *43*:1561–1566.

Bull, J.J., R.C. Vogt, and M.G. Bulmer (1982a) Heritability of sex ratio in turtles with environmental sex determina-tion. Evolution, *36*:333–341.

Bull, J.J., R.C. Vogt, and C.J. McCoy (1982b) Sex determin-ing temperatures in turtles: A geographic comparison. Evo-lution, *36*:326–332.

Bulmer, M.G. (1986) Sex ratios in geographically structured populations. Trends in Ecol. and Evol., *1*:35–38.

Burger, J., and R.T. Zappalorti (1988) Effects of incuba-tion temperature on sex ratios in pine snakes: Differen-tial vulnerability of males and females. Am. Nat., *132*: 492–505.

Burke, R.L. (1993) Adaptive value of sex determination mode and sex ratio bias in reptiles. Copeia, *1993*:854–859.

Charnier, M. (1966) Action de la température sur la sex-ratio chez l'embryon d'*Agama agama* (Agamidae, Lacertilien). Soc. Biol. Ouest Afr., *160*:620–622.

Charnov, E.L. (1982) The Theory of Sex Allocation. Princeton University Press, Princeton, NJ.

Charnov, E.L., and J.J. Bull (1977) When is sex environmen-tally determined? Nature, *266*:828–830.

Conover, D.O., D.A. Van Voorhees, and A. Ehtisham (1992) Sex ratio selection and the evolution of environmental sex determination in laboratory populations of *Menidia menidia*. Evolution, *46*:1722–1730.

Crews, D., J.J. Bull, and T. Wibbels (1991) Estrogen and sex reversal in turtles: A dose-dependent phenomenon. Gen. Comp. Endocrinol., *81*:357–364.

Deeming, D.C. and M.J.W. Ferguson (1988) Environmental regulation of sex determination in reptiles. Philos. Trans. R. Soc. Lond. [Biol.], *322*:19–39.

Deeming, D.C., and M.J.W. Ferguson (1989) The mechanism of temperature dependent sex determination in crocodilians: A hypothesis. Am. Zool., *29*:973–985.

Deeming, D.C., and M.J.W. Ferguson (1991) Physiological ef-fects of incubation temperature on embryonic development in reptiles and birds. In: Egg Incubation. D.C. Deeming and M.J.W. Ferguson, eds. Cambridge University Press, Cam-bridge, England, pp. 147–171.

Dorizzi, M., T.-M. Magnot, A. Guichard, G. Desvages, and C. Pieau (1991) Involvement of oestrogens in sexual differen-tiation of gonads as a function of incubation temperature in turtles. Differentiation, *47*:9–17.

Etchberger, C.R. (1991) Mechanistic and Evolutionary Con-siderations of Temperature-Dependent Sex Determination in Turtles. Unpublished Ph.D. dissertation, Indiana Uni-versity, Bloomington.

Etchberger, C.R., M.A. Ewert, J.B. Phillips, C.E. Nelson, and H.D. Prange (1992) Physiological responses to carbon diox-ide in embryonic red-eared slider turtles, *Trachemys scripta*. J. Exp. Zool., *264*:1–10.

Ewert, M.A. (1976) Nests, nesting and aerial basking of *Macroclemys* under natural conditions, and comparisons with *Chelydra* (Testudines: Chelydridae). Herpetologica, *32*:150–156.

Ewert, M.A., and C.E. Nelson (1991) Sex determination in turtles: Patterns and some possible adaptive values. Copeia, *1991*:50–69.

Ewert, M.A., C.R. Etchberger, and C.E. Nelson (1990) An ap-parent co-occurrence of genetic and environmental sex de-termination in a turtle. Am. Zool., *30*:56a.

Ferguson, M.W.J., and T. Joanen (1982) Temperature of egg incubation determines sex in *Alligator mississippiensis*. Nature, *269*:850–853.

Fisher, R.A. (1930) The Genetical Theory of Natural Selec-tion. Oxford University Press, Oxford, England.

Galbraith, D.A. (1986) Age estimates, survival, growth, and maturity of female *Chelydra serpentina* Linnaeus in Algonquin Provincial Park, Ontario. Unpublished M.S. the-sis, University of Guelph, Ontario.

Galbraith, D.A., M.W. Chandler, and R.J. Brooks (1986) The fine structure of home ranges of male *Chelydra serpentina:* Are snapping turtles territorial? Can. J. Zool., *65*:2623–2629.

Gutzke, W.H.N., and D. Crews (1988) Embryonic tempera-ture determines adult sexuality in a reptile. Nature, *332*:832–834.

Head, G., R.M. May, and L. Pendleton (1987) Environmental determination of sex in the reptiles. Nature, *329*:198–199.

Iverson, J.B. (1991) Phylogenetic hypotheses for the evolu-tion of modern kinosternine turtles. Herpetol. Monogr., *5*:1–25.

Janzen, F.J. (1992) Heritable variation for sex ratio under environmental sex determination in the common snapping turtle (*Chelydra serpentina*). Genetics, *131*:155–161.

Janzen, F.J., and G.L. Paukstis (1991) A preliminary test of the adaptive significance of environmental sex determina-tion in reptiles. Evolution, *45*:435–440.

Joanen, T., L. McNease, and M.W.J. Ferguson (1987) The ef-fects of incubation temperature on post-hatching growth of American Alligators. In: Wildlife Management: Crocodiles and Alligators. G.J.W. Webb, S.C. Manolis, and P.J. Whitehead, eds. Surrey Beatty and Sons, Sydney, pp. 533–537.

Kiviat, E. (1980) A Hudson River tidemarsh snapping turtle population. Trans. N. E. Sect. Wildl. Soc., *37*:158–168.

Lagomarsino, I.V., and D.O. Conover (1993) Variation in en-vironmental and genotypic sex-determining across a latitu-dinal gradient in the fish, *Menidia menidia*. Evolution, *47*:487–494.

Lance, V.A., N. Valenzuela, and P. von Hildebrand (1992) A hormonal method to determine the sex of hatchling giant river turtles, *Podocnemis expansa:* Application to endan-gered species research. Am. Zool., *32*:16a.

Lang, J.W. (1987) Crocodilian thermal selection. In: Wildlife Management: Crocodiles and Alligators. G.J.W. Webb, S.C. Manolis, and P.J. Whitehead, eds. Surrey Beatty and Sons, Sydney, pp. 301–317.

Lang, J.W., and H. Andrews (1994) Temperature-dependent sex determination in crocodilians. J. Exp. Zool., *270:*28–44.

Lang, J.W., H. Andrews, and R. Whitaker (1989) Sex determination and sex ratios in *Crocodylus palustris*. Am. Zool., *29:*935–952.

Lockwood, S.F., B.S. Holland, J.W. Bickham, B.G. Hanks, and J.J. Bull (1991) Intraspecific genome size variation in a turtle (*Trachemys scripta*) exhibiting temperature-dependent sex determination. Can. J. Zool., *69:*2306–2310.

Madsen, T., and R. Shine (1992) Sexual competition among brothers may influence offspring sex ratio in snakes. Evolution, *46:*1549–1552.

McKnight, C.M., and R.N. Gutzke (1993) Effects of the embryonic environment and of hatchling housing conditions on growth of young snapping turtles (*Chelydra serpentina*). Copeia, *1993:*475–482.

Mrosovsky, N. (1994) Sex ratios of sea turtles. J. Exp. Zool., *270:*16–27.

Mrosovsky, N., and C. Pieau (1991) Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. Amphibia-Reptilia, *12:*169–179.

Packard, G.C., M.J. Packard, and G.F. Birchard (1989) Sexual differentiation and hatching success by painted turtles incubating in different thermal and hydric environments. Herpetologica, *45:*385–392.

Pieau, C. (1978) Effets de températures d'incubation basses et élevées, sur la différenciation sexuelle chez des embryons d'*Emys orbicularis* L. (Chélonien). C.R. Hebd. Séanc. Acad. Sci., Paris (Ser. D), *286:*121–124.

Pieau, C., and M. Dorizzi (1981) Determination of temperature sensitive stages for sexual differentiation of the gonads in embryos of the turtle, *Emys orbicularis*. J. Morphol., *170:*373–382.

Schwarzkopf, L., and R.J. Brooks (1985) Sex determination in painted turtles: Effect of incubation at constant and fluctuating temperatures. Can. J. Zool., *63:*2543–2547.

Sousa, R.R., R.C. Vogt, and F.S. Gomes (1990) Nest temperatures and incubation experiments suggest that incubation temperature controls adult sex ratio in *Podocnemis unifilis* in the Rio Guapore, Rhondonia, Brazil. Proc. Ann. Meet. HL and SSAR, Tulane Univ., New Orleans, LA, 5–9 August 1990, *38/33:*89.

Tokunaga, S. (1985) Temperature-dependent sex determination in *Gekko japonicus* (Gekkonidae, Reptilia). Dev. Growth Differ., *27:*117–120.

Trivers, R.L., and D.E. Willard (1973) Natural selection for parental ability to vary sex ratio of offspring. Science, *179:*90–92.

Viets, B.E., A. Tousignant, M.A. Ewert, C.E. Nelson, and D. Crews (1993) Temperature-dependent sex determination in the leopard gecko, *Eublepharis macularius*. J. Exp. Zool., *265:*679–683.

Viets, B.E., M.A. Ewert, L.G. Talent, C.E. Nelson (1994) Sex-determining mechanisms in squamate reptiles. J. Exp. Zool., *270:*45–56.

Vogt, R.C., and O. Flores-Villela (1992) Effects of incubation temperature on sex determination in a community of neotropical freshwater turtles in southern Mexico. Herpetologica, *48:*265–270.

Webb, G.J.W. and A.M.A. Smith (1984) Sex ratio and survivorship in the Australian freshwater crocodile *Crocodylus johnstoni*. Symp. Zool. Soc. London, *52:* 319–355.

Webb, G.J.W., A.M. Beal, S.C. Manolis, and K.E. Dempsey (1987) The effects of incubation temperature on sex determination and embryonic development rate in *Crocodylus johnstoni* and *C. porosus*. In: Wildlife Management: Crocodiles and Alligators. G.J.W. Webb, S.C. Manolis, and P.J. Whitehead, eds. Surrey Beatty and Sons, Sydney, pp. 507–531.

Webb, G.J.W., S.C. Manolis, and H. Cooper-Preston (1990) Crocodile research and management in the Northern Territory: 1988–1990. In: Proc. 10th Working Meet. IUCN-SSC Crocodile Specialists Group. Gainesville, Florida, 23–27 April 1990. IUCN Publ., Gland, Switzerland, pp. 253–273.

Webb, G.J.W., S.C. Manolis, B. Ottley, and A. Heyward (1992) Crocodile management and research in the Northern Territory: 1990–1992. In: Proc. 11th Working Meet. IUCN-SSC Crocodile Specialists Group. Victoria Falls, Zimbabwe, 3–7 August 1992. IUCN Publ., Gland, Switzerland, pp. 203–246.

Wibbels, T., F.C. Killebrew, and D. Crews (1991) Sex determination in Cagle's map turtle: Implications for evolution, development, and conservation. Can. J. Zool., *69:*2693–2696.

Wilhoft, D.C., E. Hotaling, and P. Franks (1983) Effects of temperature on sex determination in embryos of the snapping turtle, *Chelydra serpentina*. J. Herpetol., *17:*38–42.

Yntema, C.L. (1968) A series of stages in the embryonic development of *Chelydra serpentina*. J. Morphol., *125:*215–252.

Yntema, C.L. (1970) Observations on females and eggs of the common snapping turtle, *Chelydra serpentina*. Am. Midl. Nat., *84:*69–76.

Yntema, C.L. (1976) Effects of incubation temperatures on sexual differentiation in the turtle, *Chelydra serpentina*. J. Morphol., *150:*453–462.

Yntema, C.L. (1979) Temperature levels and periods of sex determination during incubation of eggs of *Chelydra serpentina*. J. Morphol., *159:*17–28.