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Author(s): Edward A. Standora and James R. Spotila

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DEPARTMENT OF ZOOLOGY, IOWA STATE UNIVERSITY, AMES, IOWA 50011 (RAA); DEPARTMENT OF CHEMICAL ENGINEERING, IOWA STATE UNIVERSITY, AMES, IOWA 50011 (RCS); DEPARTMENT OF ZOOLOGY, TEL AVIV UNIVERSITY, RAMAT AVIV, TEL AVIV, ISRAEL, 69978 (RD, AA). Accepted 6 Nov. 1984.

Temperature Dependent Sex Determination in Sea Turtles

EDWARD A. STANDORA AND JAMES R. SPOTILA

Temperature dependent sex determination (TSD) has been demonstrated for loggerhead (*Caretta caretta*), green (*Chelonia mydas*), leatherback (*Dermochelys coriacea*) and olive ridley (*Lepidochelys olivacea*) turtles. Molecular mechanisms responsible for this phenomenon are unknown. Review of the literature suggests three processes which may be involved: (1) H-Y antigen may be an organizing factor for the heterogametic gonad, (2) Sex reversal may occur in genotypic males or females, (3) Specific DNA sequences such as the satellite DNA component found in some snakes may be altered in their expression or behavior as a movable or controlling element. Available data support the third hypothesis.

At Tortuguero, Costa Rica, green turtle nests on the open beach produce mainly females while those under vegetation produce 94% male hatchlings. In nests incubating at pivotal temperatures, metabolic heating results in female hatchlings at the center of the clutch and male hatchlings along the periphery. Using known pivotal temperatures and data on the temperatures of nesting beaches we compute the primary sex ratios for several populations of sea turtles. All of these are biased towards females. Finally, TSD is proposed as a major factor in the extinction of dinosaurs.

U NTIL recently it was generally assumed that all vertebrates had genetically fixed sex determination (genotypic sex determination, GSD, of Bull, 1980). However, beginning with the work of Pieau (1971, 1972, 1973, 1974, 1975a, b, 1978), and Yntema (1976, 1979) it became apparent that incubation temperature affected sex determination of freshwater turtles, with high temperatures (31 C and above) producing females and low temperatures (24 to 27 C) producing males. The importance of temperature dependent sex determination (TSD of Bull, 1980) in the biology of freshwater turtles has been elucidated by Bull and Vogt, and Wil-

hoft and their colleagues (Bull and Vogt, 1979, 1981; Bull, Vogt and Bulmer, 1982; Bull, Vogt and McCoy, 1982; Wilhoft et al., 1983). This literature has recently been reviewed by Vogt and Bull (1982).

It is now apparent that sea turtles also show TSD. Green turtles (*Chelonia mydas*) lack heteromorphic sex chromosomes (Bickham et al., 1980) and their sex is determined by incubation temperature (Miller and Limpus, 1981; Morreale et al., 1982). The loggerhead turtle (*Caretta caretta*) (Yntema and Mrosovsky, 1980, 1982; Mrosovsky and Yntema, 1980; Mrosovsky, 1982), the olive ridley (*Lepidochelys olivacea*)
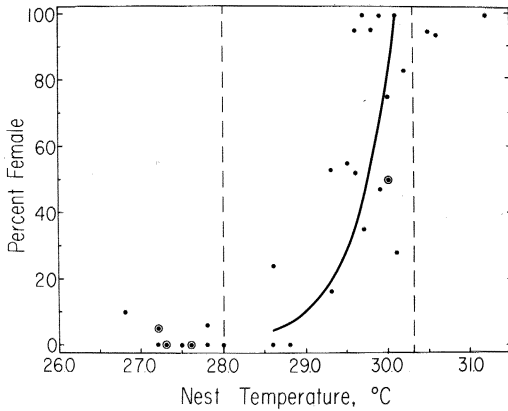
Fig. 1. Relationship between mean nest temperature during the middle third of incubation and the percent of female *C. mydas* hatchlings produced in nests on the beach at Tortuguero, Costa Rica. Temperatures below 28.0 C produced a maximum of 10% females while temperatures above 30.3 C produced a minimum of 90% females. Circled dots represent two data points.

(Morreale et al., 1982; Ruiz et al., 1981) and leatherback turtle (Mrosovsky, pers. comm.) also undergo TSD.

THRESHOLD TEMPERATURES

Threshold temperatures (pivotal temperatures) are known for two species. Yntema and Mrosovsky (1980, 1982) reported that *C. caretta* has a threshold temperature of 30 C, where approximately equal numbers of males and females are produced. Morreale et al. (1982) and Morreale (1983) found that in *C. mydas* temperatures below 28 C produce 90–100% males, temperatures between 28.5 and 30.2 C produce increasing numbers of females and temperatures of 30.5 C or higher during the middle third of incubation produce 94–100% females (Fig. 1). Miller and Limpus (1981) found that incubation at 26 C resulted in 0% females, 85.7% males and 14.3% intersexes, among hatchlings from a clutch of *C. mydas* eggs collected in Australia. Incubation at 29 C produced 90.2% females, 0% males and 9.8% intersexes, and incubation at 33 C gave 85.7% females, 0% males and 14.3% intersexes. Many questions about TSD in sea turtles remain unanswered. In this paper we will review the literature which may pertain to the molecular basis for TSD, present data on the effect of nest site on sex determination, predict primary sex ratios for several populations of sea turtles and discuss implications of TSD for dinosaur extinction.

MOLECULAR BASIS OF TSD

Molecular mechanisms responsible for TSD are unknown. The same is true for the mechanisms by which GSD works. However, a review of the literture suggests three processes that may be involved: (1) H-Y antigen may be an organizing factor for the heterogametic gonad, (2) Sex reversal may occur in genotypic males or females, and (3) Specific DNA sequences such as the satellite DNA component found in some snakes may be altered in their expression or behavior as a movable or controlling element.

The term H-Y antigen was introduced in reference to a transplantation (histocompatibility) antigen that stimulated the rejection of male skin grafts by female mice of the same inbred strain (Eichwald and Silmser, 1955; Billingham and Silvers, 1960). Several tests detect an H-Y (male specific) antigen. These include graft rejection assays, cell mediated lysis tests, and serological assays (see Muller, 1982 for a review). It has been suggested that male specific antigen(s) detected by graft rejection or cell lysis differ from serologically defined male specific antigen (called SDM, serologically detectable male, antigen) (Silvers et al., 1982). However, Koo and Varano (1981) reported that serologically detectable H-Y and H-Y antigen defined by killer cell action were similar. Thus it is not clear if there are one or several H-Y antigens and Muller (1982) suggests that the term H-Y antigen be used in all cases until it is proven that there are two or more H-Y antigens.

H-Y antigen has been highly conserved in vertebrate evolution and is usually confined to the male in species with a XX-XY mechanism of sex determination (mammals, some amphibians, and some fish; Wachtel et al., 1975, 1980; Muller and Wolf, 1979; Pechan et al., 1979). In species with a ZZ/ZW mechanism the female is the heterogametic sex and H-Y antigen is confined to females (birds, reptiles, some amphibians; Wachtel et al., 1975; Muller et al., 1979; Zaborski et al., 1979, 1980). This has led to the hypothesis that H-Y antigen directs the indifferent gonad to develop towards the mature gonad, testis or ovary, which typifies the heterogametic sex of a particular species (Wachtel et al., 1975). Recent research has demonstrated technical difficulties with H-Y serology and given conflicting results of H-Y typing (Goodfellow

TABLE 1. SUMMARY OF THE TURTLE SPECIES EXAMINED AND OF H-Y ANTIGEN TYPING PRESENTED BY ENGEL ET AL. (1981).

Family	Species	Origin	H-Y antigen	
			Male	Female
Pelomedusidae	*Pelomedusa subrufa*	Africa (Ghana)	−	+
	Pelusios subniger	Africa (Ghana)	−	+
Kinosternidae	*Kinosternon subrubrum*	America (Pennyslvania)		+
	Sternotherus minor	America (Florida)	−	+
Emydidae	Emydinae			
	Emys orbicularis	Europe (Yugoslavia)	−	+
	Pseudemys scripta elegans	America (Tennessee)		+
	Terrapene ornata	America (Carolina)	−	+
	Batagurinae			
	Chinemys reevesi	Southern China	+	−
	Cuora amboinensis	Thailand	−	+
	Siebenrockiella crassicollis	Thailand	−	+
Testudinidae	*Testudo hermanni*	Europe (Yugoslavia)	−	+
	Kinixys belliana	Africa (Kenya)	−	+
	Malacochersus tornieri	Africa (Kenya)	−	+
Platysternidae	*Platysternon megacephalum*	Southern China	−	+

and Andrews, 1982). Muller (1982) reviewed the literature and concluded that in mammals, while H-Y antigen is one important testicular differentiation factor, it is not necessarily the only and primary inducer of the testis. In nonmammalian vertebrates such as birds and amphibians H-Y antigen is also involved in the differentiation of the heterogametic gonad but again is not the only differentiation factor and is probably an estrogen dependent ovarian differentiation factor. Silvers et al. (1982) concluded that the presence of H-Y (SDM) antigen was the result and not the cause of sex differentiation. Thus H-Y antigens, detected by both transplantation and serological procedures, are frequently associated with the heterogametic sex and are probably related to sexual differentiation. However, available data indicates that H-Y antigens are not the primary cause of gonad determination.

The presence of H-Y antigen was not dependent upon the existence of morphologically distinct sex chromosomes but was associated with the heterogametic sex in amphibia (Engel and Schmid, 1981). The discovery that females in 13 of 14 species of turtles tested were H-Y positive (Zaborksi et al., 1979; Engel et al., 1981) led to the conclusion that in most turtle species a ZZ/ZW sex determining mechanism was present (Table 1). Engel et al. (1981) suggested that temperature effects on sex determination in turtles were a result of sex reversal. Zaborski et

al. (1982) incubated eggs of *Emys orbicularis* at 25–26 C, 28.5–29 C and 30–30.5 C and found that all individuals from the 25–26 C eggs were phenotypic males, all individuals from 30–30.5 C eggs were phenotypic females and 30 of 37 individuals from 28.5–29 C incubated eggs were phenotypic females, 6 were phenotypic males and one was intersex (Table 2). (Note that some of these turtles retained their sexual phenotype for 7 to 9 years, thus increasing our confidence

TABLE 2. NUMBER AND SEXUAL PHENOTYPE OF TURTLES (*Emys orbicularis*) HATCHED FROM EGGS INCUBATED AT THREE TEMPERATURE RANGES. Data from Zaborski et al. (1982). (M = Males; F = Females; I = Intersex.)

Age (months)	Number of animals				
	25–26 C	28.5–29 C			30–30.5 C
	M	M	I	F	F
3–6	38	—	1	1	23
6–12	54	4	—	17	59
12–18	29	2	—	12	13
18–24	6	—	—	—	3
24–36	5	—	—	—	2
36–48	3	—	—	—	9
48–60	11	—	—	—	9
60–72	—	—	—	—	7
72–84	—	—	—	—	2
96–108	3	—	—	—	—
Totals	149	6	1	30	127

Condition	Some consequences
BALB/cWt Y (Y-linked)	Chromosomal mosaicism; some fertile, primarily XO females, many infertile true hermaphrodites.
Y^{POS}	XY progeny develop as females or hermaphrodites.
Y^{ORB} (Y-linked)	Same as above.
Sxr (Y-linked)	XX^{Sxr} females develop as sterile males.
X^Y and Y^X (Y-linked)	XX^Y males sterile; XY^X females fertile.
Tda-1 (autosomal recessive)	XY offspring develop as females or hermaphrodites.
Dominant, autosomal, chromosome 17	Same as above.

in sex determinations made on hatchlings. Sex does not appear to change after hatching.) The blood cells of these turtles were tested for H-Y antigen with the result that of 43 phenotypic males from the 25–26 C incubation, 20 were H-Y$^+$ and 23 were H-Y$^-$. Of 21 phenotypic females from the 30–30.5 C incubation, 9 were H-Y$^-$ and 12 were H-Y$^+$. Of 14 turtles from the 28.5–29 C incubation, two phenotypic males and two phenotypic females were H-Y$^-$, while 10 phenotypic females were H-Y$^+$. Ovarian cells were H-Y$^+$ in phenotypic females whose blood cells were H-Y$^+$ as well as H-Y$^-$. Testicular cells were H-Y$^-$ in phenotypic males whose blood cells were H-Y$^-$ or H-Y$^+$. Thus Zaborski et al. (1982) concluded that the H-Y$^-$ turtles were genotypic males and H-Y$^+$ turtles were genotypic females. They hypothesized that H-Y$^+$ phenotypic males were sex reversed females and H-Y$^-$ phenotypic males were sex reversed males.

This research on H-Y$^+$ antigen and sex reversal in turtles is called into question because *Siebenrockiella crassicollis*, reported to be H-Y positive in the female by Engel et al. (1981) is in fact heterogametic in the male (Carr and Bickham, 1981). In addition, Bull (1980) demonstrated in models of the evolution of TSD from XX/XY genotypes that either the X or the Y chromosome is likely to be lost in the transition to TSD, so that there should be no trace of heterogamety left in species with TSD.

Sex reversal is a well known phenomenon in mice (see Eicher, 1982 for a review). In one case an Sxr (sex reversed) gene causes XX^{Sxr} individuals to develop as phenotyic, though sterile, males. The Sxr gene is located on the Y chromosome of male carrier mice and is transferred to the distal end of an X chromosome by crossing over during meiosis (Singh and Jones, 1982; Evans et al., 1982). Inactivation of the X chromosome causes XX^{Sxr} mice to develop as females, intersexes or sterile males (Cattanach et al., 1982; McLaren and Monk, 1982). Seven mechanisms of sex reversal are now known in mice (Eicher, 1982). Five are Y-linked and two are autosomal (Table 3). Two of the Y-linked conditions are the result of translocations during meiosis, two others involve the improper function of the testis determining gene which either sends a non-readable or mistimed signal, and the final condition involves the non-disjunction of the Y-chromosome in some cells during early embryonic development. Both autosomal mechanisms involve a change in the manner in which an autosomal gene interacts with the testis determining gene on the Y chromosome. These findings suggest that differentiation of testis from the bipotential gonad is controlled by an interaction between autosomal genes and genes on the sex chromosomes (Simpson, 1982).

The sex reversal hypothesis for TSD in turtles, proposed by Zaborski et al. (1982), is difficult to interpret in light of data on sex reversal in mice. Both the H-Y antigen studies of Zaborski et al. (1979, 1982) and Engel et al. (1981) and the heritability studies of Bull, Vogt and Bulmer (1982) suggest that there is a genetic component to sex determination in turtles. Temperature appears to act as a modifying agent affecting gene action. However, it is unlikely that TSD operates by way of activation of a sex reversal gene or genes. If one sex reversal gene were involved, then we would expect 100% of one sex when the gene was active at either low or high temperature and 50%–50% male-female at the other temperature, when it was not active. If two sex reversal genes were involved, a male inducing gene would have to be active at low temperature causing sex reversal in genotypic females and a female inducing gene active at high temperatures causing sex reversal in genotypic males. The former model does not fit the data and the latter is unduly complicated.

TABLE 4. HYPOTHETICAL SEX REVERSAL MECHANISM INVOLVING THREE CHROMOSOMES, Z, W AND AN AUTOSOMAL CHROMOSOME A. A is assumed to be temperature sensitive.

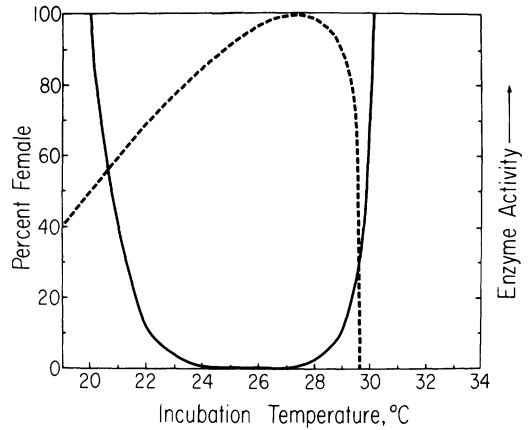| Genotype	ZZ A		ZW A	
Phenotype	H-Y	Sex	H-Y	Sex
Temperature				
Low	H-Y$^-$	male	H-Y$^+$	male
High	H-Y$^-$	female	H-Y$^+$	female

Fig. 2. Relationship between hypothetical temperature sensitive activation enzyme (dashed line) and percent of female sea turtle hatchlings (solid line) produced at different incubation temperatures.

A more likely explanation is that TSD is not a strict all or none sex reversal mechanism like those found in mice, but rather involves a modification of development of the indifferent gonad due to the differential expression of one or more specific DNA sequences whose behavior is controlled by some temperature sensitive process.

If we consider a system whereby an embryo can be either ZZA or ZWA, where Z codes for testis development, W codes for H-Y$^+$ antigen in somatic tissue and A codes for ovarian development but its expression is determined by temperature, then a ZZA individual will be male at temperatures when A is inactive and female at temperatures when A is active. It will be H-Y$^-$ in all of its somatic tissues. Likewise a ZWA individual will be male at temperatures where A is inactive and female at temperatures where A is active and it will be H-Y$^+$ in all of its somatic tissues (Table 4). We can also envision more complex systems which involve several DNA sequences, one or several of which are temperature sensitive. The major point here is that while there are insufficient data to support a particular model for the expression of TSD in turtles, a simple ZZ-ZW system with sex reversal is unlikely. This is reinforced by the fact that the turtle *Siebenrockiella crassicollis* is H-Y$^+$ in the female (Table 1) (Engel et al., 1981) but has heteromorphic sex chromosomes and is heterogametic in the male (Carr and Bickham, 1981).

Sex specific DNA sequences in turtles without heteromorphic sex chromosomes are probably scattered throughout the genome as is the case in primitive snakes. In a series of papers, Singh, Jones and colleagues (Singh et al., 1979, 1981; Singh and Jones, 1982) report that a satellite DNA sequence (Bkm) isolated from a female banded krait (*Bungarus fasciatus*) contained nucleotide sequences that had been conserved in an unusually wide spectrum of snake species and other eucaryotes. This satellite DNA hybrid-

ized to DNA on the centromeric end of the Y chromosome of male mice, to the distal end of the X chromosome of XX^{Sxr} mice, and to the W sex determining chromosome of female snakes. Primitive snakes of the family Boidae (*Eryx johni johni* and *Xenopeltis unicolor*) do not have heteromorphic sex chromosomes and these sex associated Bkm sequences are scattered in most of the macrochromosomes of these species. The relationship of these satellite DNA sequences to sex determining sequences is not clearly defined.

We hypothesize that sex specific DNA sequences occur in turtles which undergo TSD, and that these sequences are scattered through the genome and are controlled by a temperature sensitive effector molecule. A simple case would involve a DNA sequence that codes for testis development which is dependent upon an enzyme for activation. If this enzyme was inactive at low temperature (below 24 C), increased rapidly in activity between 24 and 26 C and was inactivated at a temperature between 28 and 31 C, this would explain all known examples of TSD in turtles, including Yntema's report of the production of female snapping turtles (*Chelydra serpentina*) from eggs that were incubated at 20 C during the middle third of development (Fig. 2). Enzymes are known to control DNA transcription in procaryotes and some types of viruses. This type of control is not yet demonstrated in vertebrate systems. Another case could involve hormone production. Pieau (1974) reported that temperature

TABLE 5. FACTORS OF TIME AND SPACE THAT AFFECT TURTLE NEST TEMPERATURES AND THEREFORE HATCHLING SEX RATIOS.

	Temperature → eggs → sex				
	Scale				
Factor	Large ——————————————————————————————————————→ Small				
Temporal	Centuries	Year to year	Nesting season	Incubation period	Daily
Spatial	Geographic	Local	Distance from water	Nest depth	Within nest

influenced the level of delta 5-3 beta hydroxy-steroid dehydrogenase activity in the gonads of developing *Emys orbicularis* and Zaborski et al. (1982) reported that the total levels of seven steroids was lower in ovaries at 30 C than in testes at 25 C in *E. orbicularis* embryos tested between the end of the thermosensitive period and hatching. Therefore, either enzymes and/or hormones could act as the effector molecules controlling DNA expression during TSD.

NEST SITE AND SEX DETERMINATION

Nest site affects sex determination in sea turtle embryos because it affects temperature in the nest. This involves both temporal and spatial variation (Table 5). Time effects range from those involving the middle third of development, through seasonal effects to geological time. Spatial effects range from position of eggs within the nest (for example, due to metabolic heating; Standora et al., 1982) through position of the nest on the beach, to geographic effects. We have obtained information on several of these factors for *C. mydas* and *L. olivacea*. Methodology is presented by Spotila et al. (1982) and detailed results by Morreale (1983) and Ruiz et al. (1981).

TABLE 6. PERCENTAGE OF FEMALE *Lepidocheyls olivacea* HATCHLINGS FROM DIFFERENT THERMAL CONDITIONS.

Location	Thermal condition	# nests	Total # turtles	% female
Open beach	warm	8	118	100
Shaded beach	cool	7	100	15
Tide beach	warm and cool*	1	20	10
Beach hatchery	cool	3	60	1.5
Styrofoam boxes	cold	9	164**	0.5

* Nest subjected to periodic cooling as a result of high tide inundation.
** 3 hatchlings were intersexes under this condition.

At Tortuguero, Costa Rica the beach can be divided into three thermal zones. Zone 1 (near the water) and Zone 2 (open beach up to thick vegetation) had higher temperatures at nest depth than did Zone 3 (covered by dense sea grape and cocoplum and extending into the jungle). There was no seasonal trend (from July to Oct. 2, 1980) in the soil temperatures of any of the zones. Short term fluctuations were due to changes in local meteorological conditions. Over a 24 hr period temperature variation at nest depth was less than 0.5 C, except during cooling or warming trends associated with rainy or sunny periods of several days. Six nests in Zone 1 produced 72% females, 20 nests in Zone 2 produced 87% females and 7 nests in Zone 3 produced 7% females. There was no statistically significant difference in percent females produced in Zones 1 and 2 (arcsin transformation and one way ANOVA) so these can be combined to give 83.5% female. Comparing our data with that of Fowler (1979) we computed that 71% of hatchlings emerging during the study (1977) were female.

At Playa Nancite on the Pacific coast of Costa Rica we studied TSD in *L. olivacea*. Here Ruiz found that nests in the shaded portion of the beach produced 15% females while nests in the open beach produced 100% females (Table 6). These results are reflected in nest temperatures during the middle third of development. In the open beach $\bar{x} = 33.1$ C (31.5–34.6 C) and in the shade $\bar{x} = 28.3$ C (26.4–30.0 C). Sex determination is clearly temperature dependent since nests in an artificially shaded beach hatchery ($\bar{x} = 27.4$, 25.0–28.8 C) produced 1.7% females and shaded styrofoam containers ($\bar{x} = 25.7$, 24.6–26.7 C) produced 0.6% females. Temperatures for typical sunny and shady nests are given in Fig. 3. It is impossible to predict the primary sex ratio for hatchlings from the Nancite beach because hatchability is very low and less than 1% of eggs emerge as hatchlings (Cornelius and Robinson, unpubl. report). More in-
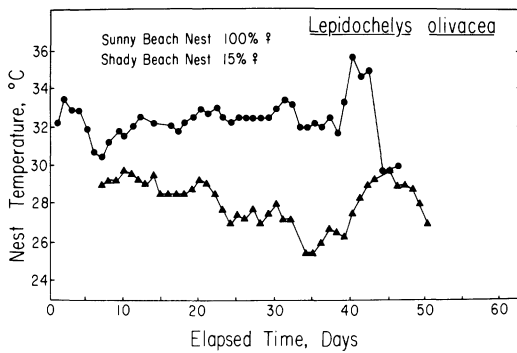
Fig. 3. A comparison of daily temperatures from two representative olive ridley (*L. olivacea*) nests from different locations on the beach at Nancite, Costa Rica (G. Ruiz, unpubl. data).
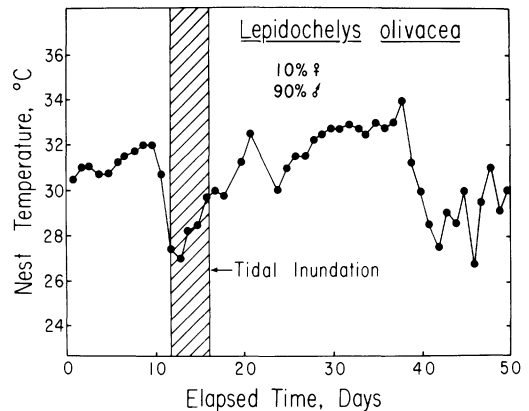

Fig. 4. Daily temperatures for an olive ridley (*L. olivacea*) nest which produced a high percentage of male hatchlings. Shaded area indicated time during which nest was submerged and subjected to cool male inducing temperatures (G. Ruiz, unpubl. data).

formation is needed on the effects of nest position on hatchability before we can predict primary sex ratio for this beach.

Factors other than temperature may influence sex determination especially in the critical temperature range that produces a mixture of females and males. For example, Bull, Vogt and Bulmer (1982) suggest that there is a genetic influence on sex determination. In addition, data from some natural nests of *C. mydas* and *L. olivacea* suggest that something other than incubation temperature is affecting sex determination. Most of the variation of sex ratio of *C. mydas* nests within the critical temperature range of 28.0 and 30.3 C can be attributed to short term variations in nest temperatures that are inadequately reflected in mean nest temperature for the middle third of development. However, one nest with $\bar{x} = 30.1$ C produced only 28% females. One nest at Nancite was low on the beach and produced only 10% females although its temperature was high ($\bar{x} = 31.6$ C, 29.7–33.1 C). This nest was subject to tidal inundation for several days at the beginning of the middle third of development (Fig. 4). The combination of lowered temperature and change in hydric environment was sufficient to induce maleness in many embryos despite high temperatures during the remainder of the middle third of development. We suggest that while temperature is the primary environmental determinant of sex in sea turtles, other factors such as osmotic stress and O_2 and CO_2 levels (Ackerman, 1981a, b) could play a role in sex determination at temperatures within the critical range where a mixture of females and males are typically produced.

PREDICTING SEX RATIOS

Because sex is determined by temperature during the middle third of development, if this temperature is known, then sex ratio of hatchlings from a nest can be predicted. If in turn this information is known for all areas of a nesting beach and all portions of the nesting season, then the overall primary sex ratio can be predicted for all hatchlings produced from that beach (see above for *C. mydas* at Tortuguero). However, the literature does not contain data on temperatures for the middle third of development for natural nests. Therefore, another approach is needed.

Mrosovsky and Yntema (1980) and Miller and Limpus (1981) report that incubation period is dependent upon incubation temperature. By computing a regression equation for the data provided by Mrosovsky and Yntema (1980) we can relate days to emergence to incubation temperature (Fig. 5). Using this equation we can predict incubation temperature for Pacific *C. mydas* eggs in beach hatchery nests in the Sarawak Islands near Borneo from data given by Hendrickson (1958). He reported incubation periods for beach hatchery nests at different seasons of the year. Using Fig. 1 we can assume that all nests with a temperature below 28 C produce 90–100% males and nests with temperatures above 30.5 C produce 90–100% females. At intermediate temperatures mixed sex ratios occur. Thus for the Sarawak Islands, most hatchery nests produce primarily females (Fig. 6). This assumes that the temperature-sex re-
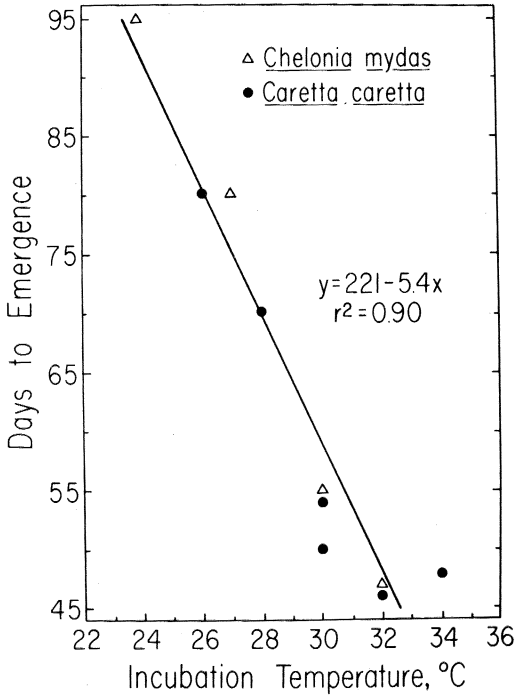
Fig. 5.   Linear regression of days required for hatchling emergence on incubation temperature, calculated from combined data for two sea turtle species (modified from Mrosovsky and Yntema, 1980).
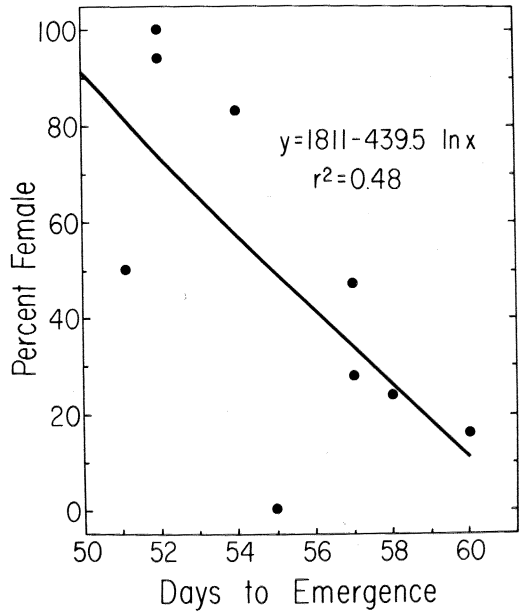
Δ Chelonia mydas
● Caretta caretta

$y = 221 - 5.4x$
$r^2 = 0.90$

Fig. 7.   Relationship between percent of female hatchlings and days to emergence for natural C. mydas nests on the beach at Tortuguero, Costa Rica.
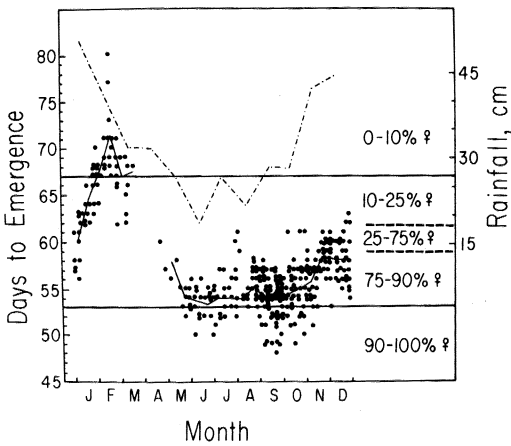
$y = 1811 - 439.5 \ln x$
$r^2 = 0.48$

Fig. 6.   Relationship between days to emergence, time of year and computed percentage of female green turtles (C. mydas) hatchlings produced in a beach hatchery on the Sarawak Islands in the South China Sea. Original data taken from Hendrickson (1958). Percent female was calculated based on temperatures obtained from regression equation from Fig. 5. Dashed line represents rainfall and solid line represents biweekly average of days to emergence.

0-10% ♀
10-25% ♀
25-75% ♀
75-90% ♀
90-100% ♀

lationship is the same for Sarawak green turtles and Tortuguero green turtles. Seasonal variation occurs, since nests laid during the monsoon season of December–January produce predominately males and those laid during April to November produce predominately females. Since hatchery nests were placed on the beach at depths similar to those of natural nests, these data are probably representative of natural nests. Thus the primary sex ratio for this population of C. mydas is biased towards females and there is an overall production of approximately 74% females for the entire year. Seasonal variation in the production of female hatchlings may also occur in the Seychelles Islands (including Aldabra) where nesting is known to occur year round (Gibson, 1979) and South Yemen where year round nesting may also occur (Hirth and Carr, 1970).

Caution must be applied in attempting to predict sex ratio of C. mydas nests from incubation times. There is a relationship between incubation period and percent female hatchlings for natural nests from Tortuguero, Costa Rica (Fig. 7). However, the r^2 value for the regression equation is only 0.48, reducing our confidence in predictions based on this relationship. This low coefficient of determination is not surpris-
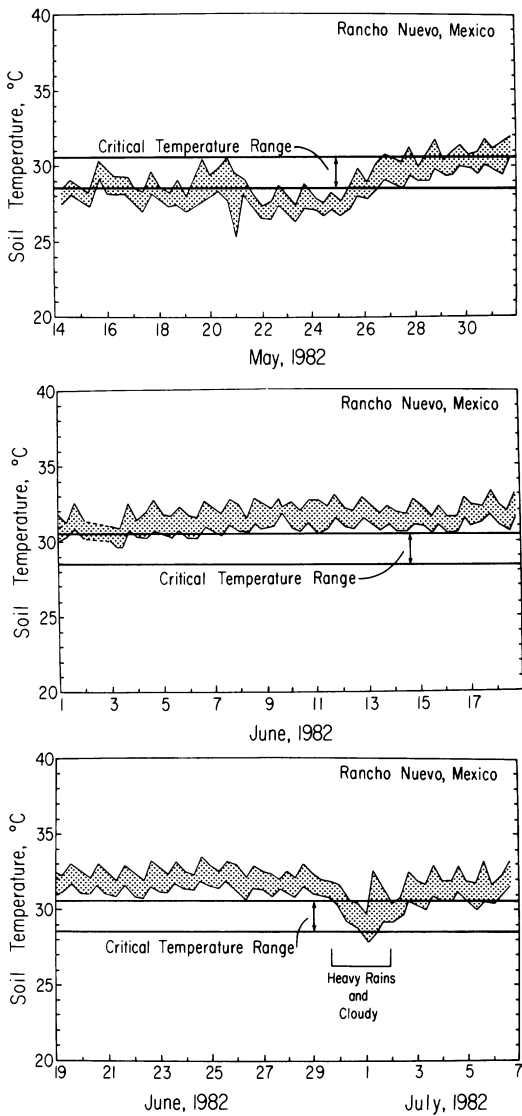
Fig. 8. Soil temperatures taken at nest depth at Rancho Nuevo, Mexico (Burchfield and Foley, unpubl.), the nesting site of Kemp's Ridley (*L. kempi*). Shaded area represents range of temperatures available from six locations at different distances from the water. Nests having temperatures during the middle third of incubation which are above the upper horizontal line would be expected to produce all female hatchlings, while those below the lower horizontal line should produce exclusively males. Nests at intermediate temperatures would have mixed sex ratios.
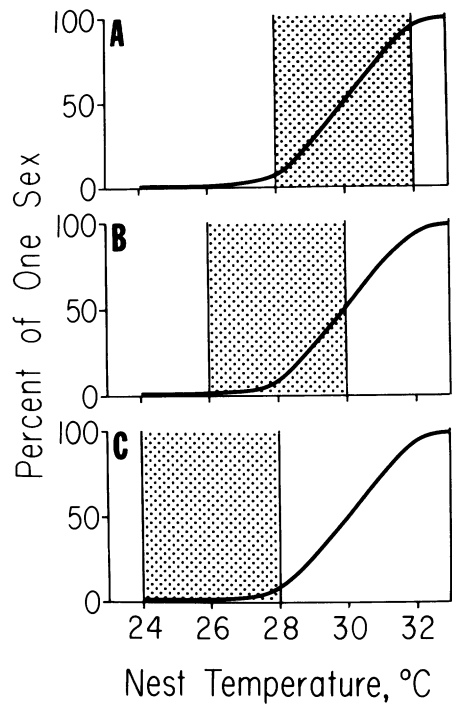

Fig. 9. Proposed relationship between sex ratio and mean incubation temperature of dinosaur eggs. Shaded area represents range of mean nest temperatures observed in nature. (A) Thermal conditions which result in offspring with equal number of each sex. (B) A 2 C downward shift in mean nest temperature results in strongly skewed sex ratios. (C) A 4 C shift in nest temperature results in almost a unisex population. Nest temperatures are chosen to represent temperatures expected to occur in upland dinosaur nests as climate cooled in late Cretaceous. Temperatures are based on data from turtle and alligator nests from tropical and temperate zone nesting sites.

ing when we consider that length of incubation is determined by the overall temperature throughout development (Fig. 6) while sex is determined by the temperature during the mid-

dle third of development. Apparently duration of incubation can be affected by temperature changes which occur in the first and last third of incubation before and after sex is determined. Despite these limitations this approach is useful in obtaining first order predictions of sex ratios for sea turtle populations. More reliable estimates can only be obtained by actually measuring nest and/or sand temperatures during incubation of natural nests. Then if the temperature/sex relationship is known, accurate predictions of sex ratios can be made.

Using temperature-sex data for the olive ridley turtle, *L. olivacea*, from Ruiz et al. (1981) and data on the temperature of the beach at

nest depth at Rancho Nuevo, Mexico from Burchfield and Foley (unpubl. report), we can make a first order prediction of the primary sex ratio of hatchling Kemp's Ridley turtles (*L. kempi*) produced under natural conditions. Fig. 8 shows the range of temperatures available at different locations on the beach for the period May 14–July 7. Superimposed are two horizontal lines encompassing the critical temperature range for *L. olivacea* which should produce mixed sex ratios. Nests that undergo the middle third of development during the latter half of May should produce a mixture of males and females with the exact sex ratio dependent upon position of a nest on the beach and changes in local meteorological conditions. By early June all nests will be at temperatures above the critical range and should produce all females. This trend continues throughout the remainder of the nesting season (Aug.) with males being produced only in those nests which are in the middle third of development during periods of heavy rains and/ or extensive cloud cover (Fig. 8). While no beach temperatures are available for the early weeks of the nesting season, they are undoubtedly lower than in May. Thus we expect that eggs laid in April produce more males and a definite seasonal change in sex ratio occurs on this, the only nesting beach for this species. Based on this analysis initial indications are that the sex ratio of *L. kempi* is also biased towards females.

TSD and Dinosaur Extinction

Temperature dependent sex determination has a profound influence on the life histories of sea turtles as well as freshwater turtles. It is also known to occur in some lizards and in alligators, *Alligator mississippiensis*, one of the closest living reptilian relatives of dinosaurs (Bull, 1980; Ferguson and Joanen, 1982). Thus, TSD probably occurred in dinosaurs and should have had a profound influence on their life history strategies as well (Ferguson and Joanen, 1982). Since dinosaur nests are known only from upland areas, we can infer that as the climate deteriorated at the end of the Cretaceous, nests would have been exposed to fluctuating and/or lower temperatures, but adults would not have been affected (Spotila et al., 1973).

A shift of 4 C in incubation temperature would dramatically alter the sex ratio and consequently the population breeding structure in one generation (Fig. 9). A shift of even 2 C would have had important demographic effects in only a few generations. Turtles and crocodilians survived because they nested near the water. As inland seas withdrew and river and lake locations changed these reptiles followed the water. Because large bodies of water moderate seasonal and daily fluctuations of temperature, eggs of turtles and crocodilians would not have been exposed to the same changes of temperature as were the eggs of dinosaurs. While primary sex ratios of present day sea turtles appear to be biased towards females, dinosaurs at the end of the Cretaceous may well have been producing generations of unisexual offspring that were the last of their species. In a system of sex determination like TSD, where environmental variability is the primary guarantee of a mixed sex ratio, fixation on a particular nesting site or type of nesting site can lead to extinction.

Acknowledgments

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DEPARTMENT OF BIOLOGY, STATE UNIVERSITY COLLEGE OF NEW YORK, BUFFALO, NEW YORK 14222. Accepted 6 Nov. 1984.