

Sex Determination and Sex Ratios of Pacific Leatherback Turtles, *Dermochelys coriacea*

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Laboratory incubation of eggs and histology of gonads indicated that the pivotal temperature for leatherback turtles nesting on the Pacific coast of Costa Rica at Playa Grande was 29.4 C and not biologically different from that determined for Atlantic leatherbacks. We calculated sex ratios of leatherback hatchlings by monitoring beach and nest temperatures for eight thermal profiles and 47 nests during the 1994-1995 nesting season. Leatherbacks at Playa Grande nest mainly in the open beach zone (86.8-90.0%) and in the middle beach section (68.6-80.9%). Sand temperature increased as the nesting season progressed and reached 30.0 C (100% female) by the middle of November. Monitored nests that produced hatchlings had temperatures above 30.0 C during the critical sex determining period. The sex of all hatchlings determined by gonad histology for 18 monitored nests ($n = 10-20$ per nest) were 100% female. Estimated sex ratio for the 1993-1994 season was 0% male:100% female, for the 1994-1995 nesting season was 6.5% male:93.5% female, and for the 1995-1996 season was 25.7% male:74.3% female. These ratios were more female biased than sex ratios reported for the past 25 years in Suriname on the Atlantic coast of South America. Gene flow between populations and the response of leatherback populations to thermally different nesting areas may be responsible for the lack of intraspecific variation in pivotal temperature.

MOST turtles examined for their sex determining mechanism have temperature-dependent sex determination (TSD) in that the temperature experienced by the embryo during the middle third of development will determine sex of the hatchling (Bull, 1980; Standora and Spotila, 1985; Ewert and Nelson, 1991). The pattern expressed in sea turtles, under both controlled laboratory experiments and natural field conditions, is low temperatures producing males, high temperatures producing females, with a narrow range of intermediate temperatures producing both sexes (Morreale et al., 1982; Spotila et al., 1987; Mrosovsky, 1994). The temperature that produces a 1:1 sex ratio under constant temperature incubation has been termed the pivotal temperature (Mrosovsky and Pieau, 1991). Mrosovsky (1980) hypothesized that females might oviposit their eggs at or near the pivotal temperature to produce a mixed sex ratio in the clutch. However, numerous studies (Vogt and Bull, 1984; Mrosovsky and Provancha, 1991; Janzen, 1994a) have shown that nest sex ratios are highly skewed (100% male or female), and pivotal temperatures have become a benchmark temperature to make comparisons between species and among populations of the same species.

Pivotal temperatures often correspond to the thermal nesting environment of the turtle population being examined and can vary both in-

terspecifically and intraspecifically. A Mojave Desert population of desert tortoises, *Gopherus agassizii*, experience high nest temperatures and have a pivotal temperature of 31.8 C, which is higher than for most species studied (Spotila et al., 1994). *Podocnemis expansa* nest in South American riverine sand bars with mean nest temperatures of 34.5 C and have the highest pivotal temperature (between 32.5 C and 34.0 C) known for any turtle (Ewert et al., 1994). These high pivotal temperatures contrast with those of painted turtles, *Chrysemys picta*, and snapping turtles, *Chelydra serpentina*, in Algonquin Park Ontario, Canada (27.5 C; Schwarzkopf and Brooks, 1985). However, across their range, pivotal temperature varies from 27.5 C to 29.0 C in painted turtles and 25.5 C to 28.5 C in snapping turtles (Ewert et al., 1994). Furthermore, the pivotal temperature of slider turtles, *Trachemys scripta*, varies from 27.0 C to 29.5 C. Modifications of pivotal temperature in different populations avoid extreme bias in sex ratios, and these differences have been attributed to factors that influence nest temperature, such as climate, nest site selection, and time of the nesting season (Ewert et al. 1994).

Interspecific and intraspecific variation in pivotal temperature for marine turtles is much narrower. Two species of sea turtles nesting on the same beach in Suriname, South America, but with different nesting seasons, have different

pivotal temperatures corresponding to the changing climatic conditions. Green turtles, *Chelonia mydas*, nest mainly during the cooler wet season and have a pivotal temperature of 28.7 C, which is 0.8 C lower than that of leatherback turtles, *Dermochelys coriacea*, which nest during the warmer dry season (Mrosovsky et al., 1984). Pivotal temperature for loggerhead turtles (*Caretta caretta*) nesting in the southeastern United States is 29.0 C (Yntema and Mrosovsky, 1980), whereas a population nesting in Australia has a pivotal temperature of 28.6 C (Limpus et al., 1985), and one from South Africa has a pivotal temperature of 29.7 C (Maxwell et al., 1988). In a review of sea turtle pivotal temperatures, Mrosovsky (1994) reported that most are clustered around 29.0–30.0 C and argued that the variation reported might reflect experimental error due to investigators using different methods to determine pivotal temperature. However, interspecific and intraspecific variation in pivotal temperature for marine turtles has been studied less extensively than in freshwater turtles.

The purpose of this study was to test for intraspecific variation in pivotal temperature for the leatherback turtle by examining a population of leatherbacks nesting on the Pacific coast of Costa Rica at Playa Grande. The pivotal temperature for leatherbacks nesting on the Atlantic coast of South America in Suriname and French Guiana is 29.5 C (Rimblot et al., 1985; Rimblot-Baly et al., 1987). Vegetation surrounding these nesting colonies is tropical moist forest with an average of 2.0–3.0 m precipitation annually with considerable rain even during the drier parts of the year (Schulz, 1975). In contrast, areas surrounding Playa Grande are dry tropical deciduous forests under the Holdridge life zone system and receive an average of 1.0–1.5 m precipitation annually (Holdridge, 1967; Coen, 1983). Vegetation surrounding these two nesting areas indicates long-term differences in climate which would permit selection for intraspecific variation in the pivotal temperature between these populations. Therefore, leatherbacks nesting at Playa Grande might have a higher pivotal temperature than those nesting on the Atlantic beaches. We measured the pivotal temperature of leatherback embryos and the sex of emerging hatchlings at Playa Grande during the 1994–1995 nesting season, concentrating on temporal and spatial variation in sex ratio. Then, we used temperature data from other nesting seasons to calculate sex ratios of hatchlings during these years.

MATERIALS AND METHODS

Study site.—Playa Grande is a 3.5-km long stretch of nesting beach located in the Province of Guanacaste on the Pacific coast of Costa Rica (10°20'N, 85°51'W). Areas surrounding Playa Grande receive the majority of their precipitation in the wet season months of August to October. Historically, Guanacaste is the warmest and driest region in Costa Rica with a pronounced dry season that starts in November and lasts until April. This dry season encompasses the majority of the nesting season and is characterized by strong offshore Papagayo winds (10–30 km/h) from January to March (Coen, 1983).

Female leatherbacks come to Playa Grande during the entire year with large numbers from November to February and a peak nesting in December. Urban development occurs at both the northern and southern ends of Playa Grande, and high tides wash over most of the beach at the northern 1.6 km and the southern 0.5 km. Both of these factors influence nest distribution. Playa Grande is the largest of three adjacent nesting beaches and has the highest number of nesting leatherbacks. These beaches are protected as part of Parque Marino Las Baulas. This is a major step in the conservation of Pacific leatherbacks since this area supports the fourth largest nesting colony of leatherbacks in the world and the largest remaining in the Pacific Ocean (Spotila et al., 1996).

Sex determination, pivotal temperature, and sex ratios.—We constructed eight constant-temperature incubators from 5.0-cm thick styrofoam boards with dimensions of 60 cm by 50 cm by 35 cm (l × w × h). A single plastic tupperware water tub placed in the bottom of the incubators served as a heat sink and a submersible aquarium heater, and an air stone provided a heat source and water circulation. A mercury switch contact thermometer placed in the water tub was the temperature-control device (see Lang et al., 1989). We set six incubators at 28.0–33.0 C (± 0.2 C) at one-degree intervals, one at 29.5 C, the Atlantic pivotal temperature, and one at 30.5 C. Lack of development at 33.0 C caused us to reset this incubator at 31.5 C.

We collected eggs from “doomed” leatherback nests laid below the high tide mark from Playa Ventanas. Playa Ventanas was located directly north of Playa Grande, had no berm, and was washed over during the spring tides. We collected 20–30 eggs from nests as they were being laid and immediately transported eggs to our laboratory 1 km from the beach. We divided

eggs randomly among the different incubators, to avoid clutch effects, and placed them in plastic trays filled with beach sand from Playa Ventanas. We inserted 24-gauge copper-constantan thermocouples (Cu-Cn) into the sand touching the eggs. We recorded temperatures (± 0.2 C) three to four times daily using a Bat 12 thermocouple reader.

We sacrificed hatchlings by decapitation, dissected them for kidneys and gonads, preserved tissues in Bouin's solution, embedded them in paraffin, sectioned at 7 μm , and stained with hematoxylin/eosin for histological determination of sex (Spotila et al., 1987). Sexing criteria followed Morreale et al. (1982), and Wibbels et al. (1991).

Numbered markers divided Playa Grande into 50-m sections during the 1994–1995 nesting season. Spring tides clearly separated the beach into three horizontal sections designated as north beach from 0–1.6 km, which was washed over during the highest tides; midbeach from 1.6–3.0 km, which was protected from the spring tides, and south beach from 3.0–3.5 km, which was similar to the north beach and was washed by the highest tides. Females were tagged and nest position recorded (marker number and zone) for all nesting females (for a complete description of beach monitoring, see Steyermark et al., 1996).

Nest temperatures and beach temperatures measured using 24-gauge Cu-Cn thermocouples allowed us to determine sex ratios of hatchlings produced at Playa Grande. We did a survey of beach temperatures every 1 m from the water to the vegetation to determine whether temperatures varied in different areas. Based on temperature differences and following Spotila et al. (1987) and Leslie et al. (1996), we separated the beach into three vertical zones available to a nesting female that included zone 1, below the high tide mark; zone 2, open beach; and zone 3, vegetation. Nesting ecology of leatherbacks at Las Baulas was reported by Steyermark et al. (1996) and Chaves et al. (1996). Ten thermal profiles were constructed from wooden meter sticks with thermocouples placed at 10, 25, 50, 75, and 100 cm. We buried these on different parts of Playa Grande to determine the different thermal zones present and recorded their temperatures every day using a Bat 12 thermocouple reader for the entire nesting season. Four were in the north section (two in zone 3, two in zone 2); four were in the midbeach section (two in zone 3, two in zone 2); and two were in the south beach section (one in zone 3, one in zone 2).

We measured from the bottom of the nest

chamber and from the top of the eggs to the beach surface using a wooden meter stick to determine nest depth (cm). We placed thermocouples in the center of leatherback nests when females were laying eggs and recorded nest temperatures with a Bat 12 thermocouple reader every day for the entire incubation period. We monitored nest temperatures of 47 nests during the 1994–1995 nesting season, of which 20 were laid in November, 20 were laid in December, and seven were laid in January. Nine of the monitored nests were in the north section, 31 were in the midsection, and seven were in the south beach section. Only one nest was in zone 1 and one in zone 3, and the other 45 were in zone 2. A thermocouple placed 1 m to the side of each monitored nest at nest depth served as a control and determined the extent and beginning of metabolic heating. Five nests had five thermocouples placed in them (top-center-bottom of the nest, left and right side of the center on the periphery of the clutch) to test for within-nest variation in temperature and sex ratio. Nest and profile temperatures were read once a day since three 24-h temperature scans, in which profile temperatures at nest depth and several nest temperatures were measured every 2 h, showed diurnal temperature variation of only 0.3 C. We analyzed temperature data from the 1993–1994 and 1995–1996 nesting seasons to make sex ratio comparisons between years.

We used super ANOVA software to analyze temperature data by performing a repeated measures analysis of variance (ANOVA) on the two beach zones, three beach sections, four months (November–February using two-week mean temperatures), and three profile depths (50, 75, and 100 cm). Zone and beach section were the between individual treatments, whereas time and depth were the within individual treatments.

RESULTS

Pivotal temperature.—The pivotal temperature for leatherback turtles nesting at Playa Grande was 29.4 C (Fig. 1). Eggs incubated below 29.0 C were 100% male, whereas those above 30.0 C were 100% female. Eggs incubated at 29.5 C produced a mixed sex ratio of 40% male:60% female. Hatching success at 28.0, 29.0, 30.0, 30.5, 31.0, 31.5, 32.0, and 33.0 C was 73.9, 45.5, 63.6, 69.6, 45.5, 54.6, 36.4, 50.0, and 0.0%, respectively. Histological examination of the gonads showed a clear difference between the sexes. The ovary, testis, and oviduct were attached to the kidney by a narrow stalk of connective tissue. Female ovaries had a thick outer cortical

$12\text{mm} = 25^\circ$
 $2.5\text{mm} =$

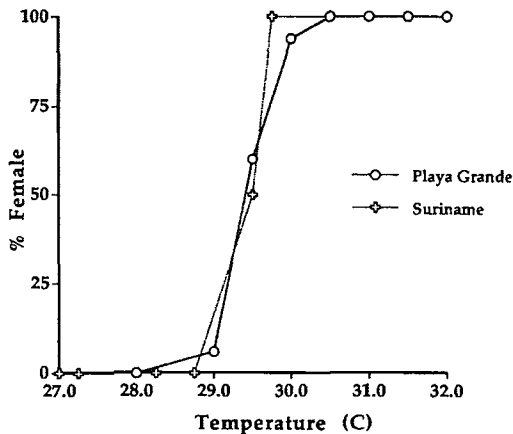


Fig. 1. The two temperature versus percent female curves for Atlantic and Pacific leatherback populations are virtually identical in pivotal temperature and overall shape. There is little intraspecific variation in the sex-determination response to temperature in these populations.

zone that was composed of cuboidal epithelial cells. The oviduct was well developed in females with a thick cortex of cuboidal epithelial cells and a large lumen lined with dark staining cells. The testis was a smooth, oval-shaped structure enclosed in a layer of simple squamous epithelium. The oviduct was either absent or degenerate in male leatherback hatchlings.

Beach temperatures, nest temperatures, and sex ratios.—Of the original 10 thermal profiles placed on Playa Grande during the 1994–1995 nesting season, one in the north section (zone 2) and one in the midbeach section (zone 3) were stolen. Data from the eight surviving thermal profiles indicated that time ($P = 0.0001$, $F = 199.38$, $df = 6,24$) and depth ($P = 0.0027$, $F = 36.28$, $df = 2,24$) had statistically significant effects on temperature, whereas zone ($P = 0.1017$, $F = 8.362$, $df = 1,24$) and section ($P = 0.2760$, $F = 2.622$, $df = 2,24$) did not. The two-way interactions of time with depth, zone, and section were statistically significant as was the three-way interaction of time \times zone \times section ($P = 0.0070$, $F = 4.533$, $df = 12,24$).

The nesting ecology of leatherback females greatly reduced the number of thermal microhabitats experienced by the eggs. Average depth to the middle of the nest was 70.1 cm ($n = 80$ nests, $SD = \pm 10.7$ cm), and average distance from the bottom of the nest to the top of the egg mass was 18.2 cm. The majority of nests experienced sand temperatures that were intermediate between those at 50- and 75-cm depths. Most females nested in zone 2 at the midbeach

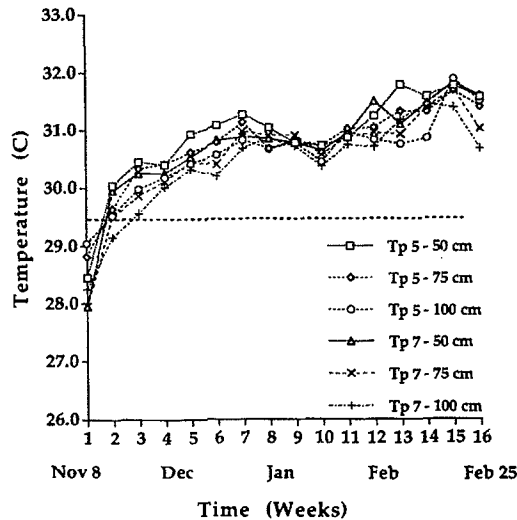


Fig. 2. Weekly mean sand temperatures from two thermal profiles in zone 2 at the midbeach section of Playa Grande, Costa Rica, during the 1994–1995 nesting season. Temperatures increased as the nesting season progressed and were lower at deeper depths. Temperatures quickly rose above pivotal as the wet season ended and dry season began. Dashed line represents the pivotal temperature of 29.4 C.

section. During the 1994–1995 nesting season, females laid 8.3% of the nests in zone 1, 86.8% in zone 2, and 4.9% in zone 3. Leatherbacks repeated this pattern during the 1995–1996 season with 7.4, 90, and 2.6% of the nests being laid in zones 1, 2, and 3, respectively. Location of zones for nests was not recorded for the 1993–1994 nesting season. During these three nesting seasons, 21.2, 24.4, and 11.4% of nests were in the north beach section; 75.5, 69.0, and 80.9% of nests were in the midbeach section; and 3.3, 7.0, and 7.7% of nests were in the south beach section. Of 244 hatched nests for which we recorded zone and beach section during the 1994–1995 nesting season, 79.1% were from the midbeach section, whereas 11.9 and 9.0% were from the north and south sections, respectively. All of these nests were in zone 2 with no hatched nests observed in zone 1 or 3.

Since 86.0–90.0% of nests were in zone 2, we restricted further analysis of beach temperatures to those from zone 2 at nest depth (50 and 75 cm temperatures). Time ($P = 0.0001$, $F = 135.37$, $df = 6,12$) and depth ($P = 0.0106$, $F = 358.52$, $df = 1,12$) had statistically significant effects on temperature (Fig. 2), whereas section ($P = 0.1915$, $F = 13.14$, $df = 2,12$) did not. The two-way interactions of time with depth and section were also statistically significant.

Nests laid in early November quickly rose

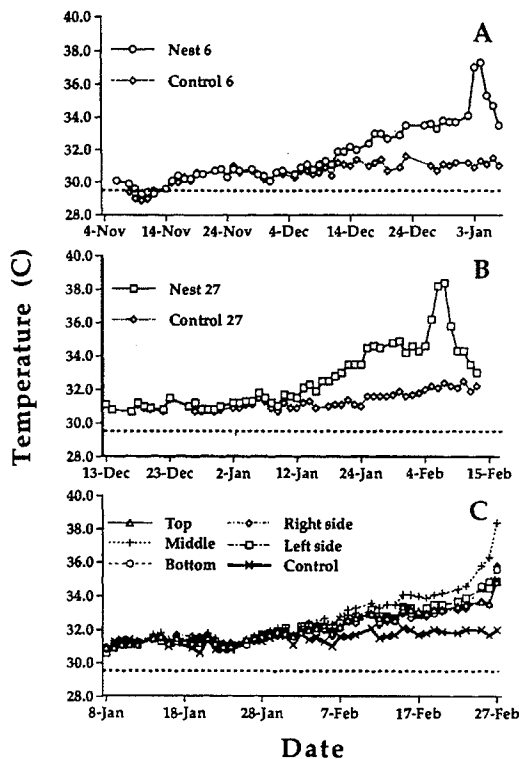


Fig. 3. Nest and control temperatures for three leatherback turtle nests on Playa Grande, Costa Rica, during the 1994-1995 nesting season. Initial nest and control temperatures increased and incubation times decreased as the season progressed. Metabolic heating raised nest temperatures up to 7.0 C higher than that of controls and occurred as early as the middle third of incubation. Dashed line represents the pivotal temperature of 29.4 C.

above 30.0 C and increased in temperature throughout development (Fig. 3A). This reflected the general increase in sand temperatures at nest depths. Nests laid from late November to January had initial temperatures that ranged from 30.0-32.0 C (Fig. 3 B-C). Temperatures of nests closely resembled control temperatures until late in the middle third of development when nest temperatures increased, as a result of metabolic heating, and reached 4.0-8.0 C above control temperatures just prior to hatching. Diurnal variation in nest temperature measured every 2 h several times during incubation was approximately 0.3 C, whereas intranest variation in temperature was 0.3-0.4 C until the middle of incubation when intranest variation reached 1.0 C and up to 4.0 C near the end of development (Fig. 3C).

Mean nest temperatures during the middle third of development (critical sex-determining period), for all but one monitored nests that

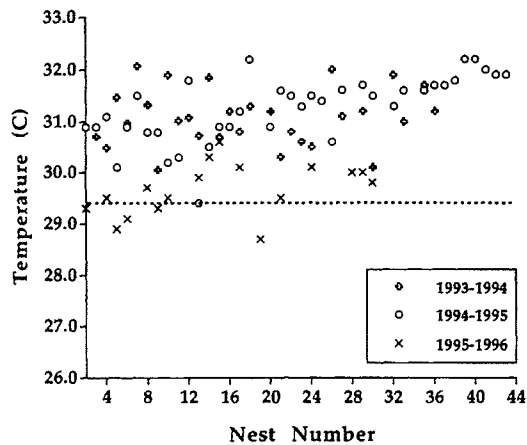


Fig. 4. Mean nest temperatures during the middle third of development, when sex is determined, of leatherback turtle nests laid on Playa Grande, Costa Rica, during the three nesting seasons. From 1993-1995, all nests laid from November to February were 100% female reflecting the drought in the Guanacaste region during this time. Higher rainfall during the 1995-1996 season resulted in temperatures mainly around the pivotal temperature and a higher percent male sex ratio during this season. Dashed line represents the pivotal temperature of 29.4 C.

produced hatchlings during the 1994-1995 nesting season were above 30.0 C (Fig. 4). Histological examination of gonads of hatchlings taken from 18 monitored nests ($n = 10-20$) during the 1994-1995 nesting season indicated that hatchlings were 100% female.

Steyermark et al. (1996) reported that on average 13% of nests are laid in October at Playa Grande. If we designate half of these nests as 100% male, then the estimated sex ratio produced at Playa Grande during the 1994-1995 nesting season was 6.5% male:93.5% female. We calculated this by multiplying the sex ratio of each two-week period by the nesting frequency during that month. This was the most conservative estimate of percent female since there was no data on beach temperatures before November.

Data from zone 2 midbeach thermal profiles at nest depth from the 1993-1994 nesting season (Williams, 1995) gave temperatures above 30.0 C from November to February (Fig. 5). Mean temperatures during the middle third of development from 30 monitored nests during this season were all above 30.0 C (Fig. 4). The 1993-1994 nesting season produced 100% females from the middle of October to the end of the nesting season. If we assumed 13% of nests were laid in October and all of these nests were 100% female, since their critical sex-deter-

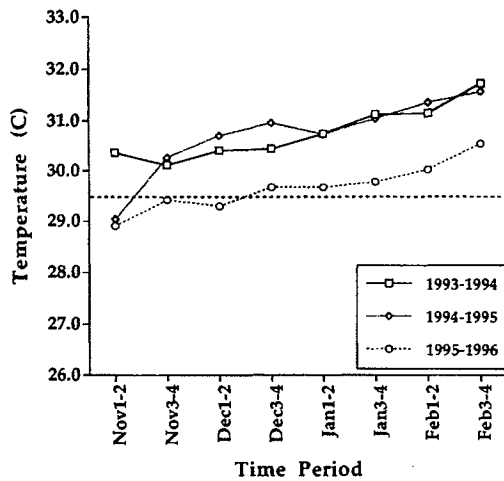


Fig. 5. Two-week mean temperatures from the midbeach section of Playa Grande at nest depth for three nesting seasons. The 1993-1994 and 1994-1995 nesting seasons were under drought conditions in Guanacaste and resulted in 100% female sex ratios from November to February. Rainfall during the 1995-1996 season resulted in a mixed sex ratio. Dashed line represents the pivotal temperature of 29.4 C.

mining period was in the beginning of November, then the estimated sex ratio for the 1993-1994 nesting season was 0.0% male:100% female.

The 1995-1996 season was cooler due to rainfall even into the month of January. Zone 2 midbeach sand temperatures at nest depth and mean nest temperatures during the middle third of development for 15 monitored nests were between 29.0 C and 30.0 C for the majority of the nesting season (Figs. 4-5). We calculated an estimated sex ratio of 81.8% female and 18.2% male using temperature data from November to the end of the nesting season. If we assumed 13% of nests were laid in October and that three-quarters of these would be producing 100% male hatchlings, then the estimated sex ratio for the 1995-1996 season was 25.7% male:74.3% female.

DISCUSSION

Pivotal temperature.—The pivotal temperature of 29.4 C for leatherbacks at Playa Grande, Costa Rica, was not biologically different from the 29.5 C value found by Rimblot et al. (1985) and Rimblot-Baly et al. (1987) for leatherbacks nesting on the Atlantic coast in Suriname and French Guiana (Fig. 1). It fell within the range of 29.2-30.4 C for the shift from male to female in leatherback hatchlings at Rantau Abang, Ma-

laysia (Chan and Liew, 1995). It also fell within the range of 29.0-30.0 C for pivotal temperatures of most sea turtles (Mrosovsky, 1994). The overall shapes of the two temperature versus hatchling sex curves for leatherbacks were almost identical. The differences in percent female from 29.0-30.0 C may reflect differences in sample size between the experiments. Rimblot et al. (1985) and Rimblot-Baly et al. (1987) had three to four hatchlings per temperature, whereas this study had eight to 19 hatchlings per treatment. This information should be incorporated into any conservation programs that manipulate leatherback eggs (but see Frazer, 1992). We rejected the hypothesis of an elevated pivotal temperature reflecting the climatic differences between the Atlantic and Pacific nesting areas. Equal pivotal temperatures between Atlantic and Pacific populations might be the result of the presence of gene flow between the two populations and/or similar thermal nesting environments between these geographically separated nesting areas.

Dutton et al. (1996) showed that the mtDNA sequence divergence between Pacific and Atlantic leatherback populations is 0.0081. This value is low compared with divergence values (0.071-0.074) for green turtles between the two oceans and is similar to that found between different nesting beaches for other sea turtle species in the same ocean basin. This implies gene flow or recent evolutionary separation between Pacific and Atlantic leatherback populations. Leatherback turtles can travel great distances and have been found in Arctic waters at 70°N latitude. It is not known whether individuals move between ocean basins; however, given their migratory abilities and suspected two- to three-year remigration times between nesting seasons, it is possible.

Ewert et al. (1994) reported differences in pivotal temperature of up to 3.0 C for several wide-ranging freshwater turtle species. The large intraspecific variation in pivotal temperature in these turtles may be in part due to lack of gene flow. Walker et al. (1995) examined population genetic structure and divergence in musk turtle (*Sternotherus minor*) populations in the southeastern United States using mtDNA analysis. The mtDNA of almost every population, even those within the same river basin, could be distinguished from all others resulting from lack of gene flow between populations. If there is more gene flow between leatherback populations than in other sea turtles and in freshwater turtles, intraspecific variation in pivotal temperature in leatherbacks might not be expected. However, another crucial factor is the

selective pressure imposed on leatherback females at Playa Grande by their thermal nesting environment. Is Playa Grande truly thermally different from Atlantic nesting beaches?

Beach temperatures, nest temperatures, and sex ratios.—Differences in beach temperatures with depth and time (Fig. 2) were due to the heating effect of increased solar radiation as the dry season began in mid-November. There was a thermal lag at increased depth proportional to the thermal conductivity, heat capacity, and thermal diffusivity of the beach sand (Rose, 1966; Spotila et al., 1987). Daily temperature variation was negligible at nest depth because nest depths were below the thermal damping depth for wet sand (Campbell, 1977). Spotila et al. (1987) reported a similar phenomenon for the beach at Tortuguero on the Atlantic coast of Costa Rica.

The biological effect of spatial factors (different beach zones and sections) on sex determination was decreased because leatherbacks nested predominately in zone 2 in the midbeach section. Females avoided nesting in the vegetation at Playa Grande as did leatherbacks in Suriname and Tortuguero (Mrosovsky et al., 1984; Whitmore and Dutton, 1985; Leslie et al., 1996). Playa Grande leatherbacks placed only 7–8% of nests in zone 1 or the doomed nest zone, as compared with Suriname and Saint Croix (Mrosovsky, 1983; Eckert, 1987; Godfrey et al., 1996) where up to 60% of the nests were washed away by tides and waves. If Playa Grande is truly warmer than Atlantic nesting colonies, there has been no selection for females to nest in cooler microhabitats. Playa Grande leatherback females appear to have adjusted their nesting strategy to one that optimizes hatching success by placing nests in areas protected from the daily and spring high tides. This is unlike leatherbacks at Saint Croix and Suriname, which scatter their nests (Eckert, 1987) because the beaches are more dynamic and thus less stable and “predictable.”

Zone 2 sand temperatures at nest depth increased as the nesting season progressed (Fig. 2). Increasing temperatures apparently caused the end of the nesting season in February. Decreased survivorship of nests under high temperature conditions may select against nesting in March and April. Leatherback eggs did not develop at 33.0 C in the incubators. This may be the upper temperature tolerance limit for early embryonic development. There appears to be an increase in temperature tolerance during embryonic development since leatherback eggs did survive higher temperatures (> 38 C) during the final third of development due to met-

abolic heating (Fig. 3). These were some of the highest nest temperatures recorded for turtles and were similar to those of softshell turtles, *Apalone mutica* (Plummer et al., 1994).

High nest and beach temperatures during the 1993–1994 and 1994–1995 seasons and extreme female-biased sex ratios, reflected the last two years of a six-year drought in the Guanacaste region (D. H. Janzen, pers. comm.). Based on temperatures similar to 1993–1995, female-biased sex ratios could be extrapolated back four more years resulting in a six-year female-biased sex ratio. Factors such as nest depth and temperature gradients in the clutch, which affected sex ratios in green turtle nests (Standora et al., 1982), had negligible effects on sex ratio in the 1993–1994 and 1994–1995 seasons. There were temperature gradients in leatherback clutches (Fig. 3C), but all temperatures regardless of position were above 30.0 C for the entire incubation period. Metabolic heating occurred during the middle third of development (Fig. 3) but did not affect sex determination since nests began development at temperatures that produced 100% female (Fig. 1). Nest depth and intranest temperature gradients probably had a greater effect on sex ratio during years when beach temperatures hovered around the pivotal temperature as in the 1995–1996 season. During the 1995–1996 season, high amounts of rainfall, even into January, accounted for the lower beach and nest temperatures and higher percent male sex ratio (Figs. 4–5).

Data on beach temperatures, nest temperatures, and sex ratios from Suriname indicated that female hatchlings are produced predominately at the beginning and end of the nesting season, whereas males are produced in greater numbers during the middle of the season. Beach temperatures did not become high enough to produce 100% females until July, after the height of the nesting season was passed. Mrosovsky et al. (1984) calculated an overall sex ratio of 51.0% male:49.0% female from 11 years of rainfall data. From 1979 to 1993, hatchling leatherbacks were 35–70% female in different years with an overall sex ratio of 46.6% male:53.4% female (Godfrey et al., 1996). Sex ratio was never as highly skewed in the 25 years at Suriname as that seen for the 1993–1994 and 1994–1995 seasons at Playa Grande. In 1993, Suriname produced 69% female, whereas Playa Grande produced 100% female hatchlings. Different nesting populations of leatherback turtles produce greatly different sex ratios in the same year. In 1990, the Rantau Abang leatherback Sanctuary in Malaysia produced 100% female hatchlings (Chan and Liew, 1995). We do

not know whether this female bias occurred in other years at this beach.

More data are needed to compute sex ratios for Costa Rican leatherbacks over a longer time scale. From 1993–1996, there was a strong female-biased sex ratio, much like that seen for leatherbacks in Malaysia (Chan and Liew, 1995) and loggerhead sea turtles in Florida (Mrosovsky and Provancha, 1991). Females move between the three nesting beaches (Playa Grande, Ventanas, Langosta) in the Parque Marino Las Baulas (Chaves et al., 1996) and perhaps to Playa Naranjo located 45 km north. Interestingly, migrations of up to 120 km occur in leatherbacks nesting in the Caribbean Sea (Eckert et al., 1989; Keinath and Musick, 1993). Therefore, temperature data are needed from all of these beaches to better understand the long-term trends in sex ratios of Costa Rican leatherbacks.

Climate change.—Three ways in which leatherback females could be selected to respond to geographic and temporal differences in climate are by a change in their nest site selection to cooler or warmer microhabitats, a change in their pivotal temperature, or a change in the time of the nesting season. The first two possibilities do not occur. Playa Grande females do not adjust their nest site selection to cooler areas (zone 1 and 3) in response to the warmer Guanacaste climate. They do the opposite and concentrate nests in zone 2. Furthermore, there is no indication that nest depth differs between populations (Godfrey et al., 1996). Likewise, pivotal temperature is the same for Playa Grande leatherbacks as elsewhere. Selection does act on the timing of the nesting season in leatherbacks. Playa Grande leatherbacks nest from November to February, whereas Atlantic leatherbacks in Suriname and French Guiana nest predominately from April to July (Schulz, 1975; Fretey and Girondot, 1987). Malaysian leatherbacks nest from May to October (Chan and Liew, 1995; for a review of leatherback nesting seasons, see Hirth, 1980). Apparently leatherback turtles have responded to geographic differences in climate by changing the time of the nesting season through selection for those months that optimize hatching success and development.

The colonization of Pacific Costa Rican beaches some time in the past 2–3 million years, occurred late in the evolutionary history of *Dermochelys coriacea*. Leatherbacks are known to have the lowest nest site fidelity of any sea turtle (Eckert et al., 1989; Leslie et al., 1996) and demonstrate different nest site selection on differ-

ent beaches, along with the ability to change nesting season. This has allowed leatherbacks to colonize many tropical and subtropical beaches without changing pivotal temperature. Janzen (1994b), suggests that turtles are unable to evolve rapidly enough to counteract the negative consequences of rapid global temperature change. Whether the flexibility in timing of reproduction seen in different populations of leatherbacks in response to geographic differences in climate is great enough to also allow this species to adapt to rapid global climate changes remains unknown. However, the strongly female-biased sex ratios on Playa Grande for several years and in Malaysia are cause for concern and indicate the need for long-term estimates of sex ratios for leatherback beaches throughout the world.

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