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# 11 Mixed and Uniform Brood Sex Ratio Strategy in Turtles : *The Facts, the Theory, and Their Consequences*

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## CONTENTS

11.1	Introduction.....	279
11.2	Unisex or Mixed Sex Ratio Strategy in a Nest: Which is Best?.....	280
11.3	Review of the Proportion of Unisex Nests from Field Studies.....	283
	11.3.1 Sampling Requirements.....	283
	11.3.1.1 Fair Spatial Sampling.....	283
	11.3.1.2 Fair Temporal Sampling.....	283
	11.3.1.3 Accurate Classification of Sex.....	284
	11.3.2 Data from the Literature.....	284
	11.3.3 Analyses of the Sex Ratio Data.....	290
11.4	Global Warming and the Fate of TSD Turtles.....	292
11.5	Unisex versus Mixed Broods and the Evolution of TSD.....	295
	References.....	296

## 11.1 INTRODUCTION

Temperature-dependent sex determination (TSD) occurs when males or females are differentially produced according to the incubation temperature (Bull, 1983). Since the discovery of TSD in a squamate by Charnier (1966), this pattern of sex determination has been described in various reptiles: all crocodylians (Deeming, 2004), tuataras (Nelson et al., 2004), some squamates (Harlow, 2004), and 64 out of the 79 studied turtle species (Ewert et al., 2004). Other reptile species exhibit genotypic sex determination (GSD), where sexual phenotype is independent of embryonic incubation temperature. GSD in reptiles is sometimes linked with heteromorphic sex chromosomes, with males or females being the heterogametic sex. However, many species with GSD do not exhibit strong differentiation of sex chromosomes. Overall, the presence of dimorphic sex chromosomes is not necessarily mutually exclusive of TSD, as has been demonstrated in various amphibians (Chardard et al., 2004) and one lizard (Shine et al., 2002).

Three distinct patterns of TSD are observed in reptiles but only two are present in turtles. Pattern TSD Ia or MF is observed in many turtles and is characterized by the production of males at lower incubation temperatures and females at higher temperatures (Ewert et al., 1994). For pattern TSD Ib or FM, females are produced at lower incubation temperatures and males at higher temperatures. This pattern is observed in some lizards (Viets et al., 1994) but was originally described in

crocodilians. The recent availability of more complete data for some crocodile species has revealed that many species actually exhibit TSD II or FMF, where females are produced at low and high temperatures and males at intermediate ones (Lang & Andrews, 1994). Note that some turtle species also exhibit pattern II (Ewert et al., 1994). The relationships among these patterns are subject to debate. It has been proposed that FMF is the general pattern for reptiles and that FM or MF are simply observed because extreme incubation temperatures have not been adequately studied or because sufficiently lower or higher incubation temperatures are not conducive to successful incubation (Pieau et al., 1995).

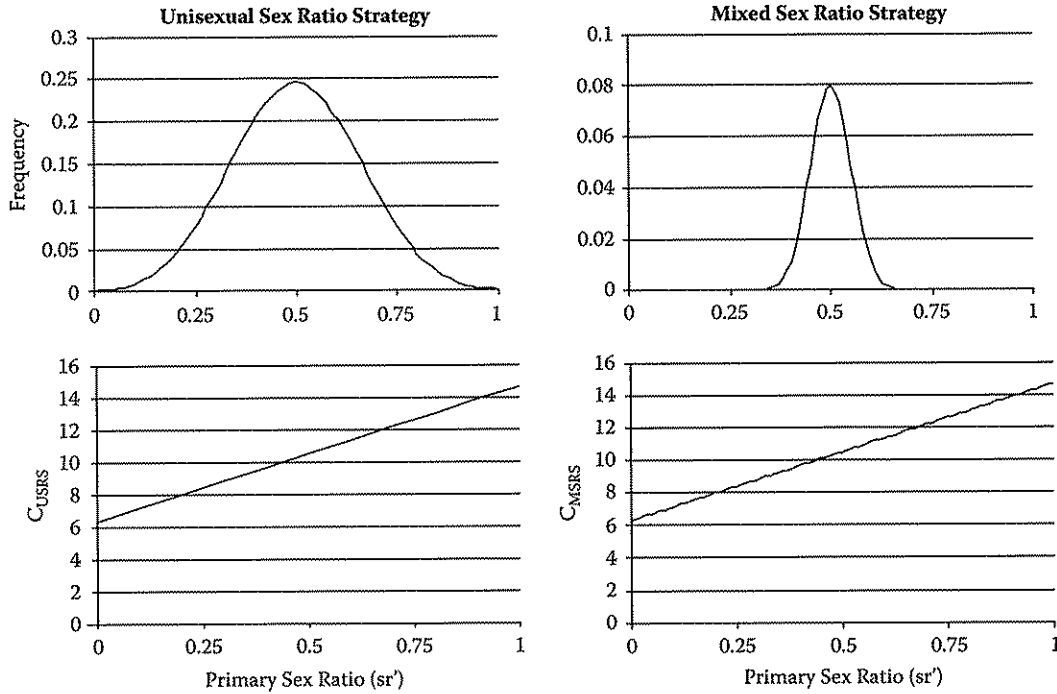
The precise timing when sex determination is sensitive to temperature during development has been studied in various reptiles. The timing is always linked with the first stages of gonadal development until the end of the second third of development (Pieau & Dorizzi, 2004). This homogeneity among various reptilian orders as well as recent phylogenetic analyses (Janzen & Krenz, 2004) suggest a common origin for TSD in this class.

The selective forces explaining the prevalence of TSD in turtles remain elusive. The most-studied hypothesis was formulated by Charnov and Bull (1977). According to this theoretical model, environmental sex determination (ESD) should be favored over GSD when offspring develop in a spatially heterogeneous (patchy) environment for one parameter, this parameter influencing the fitness of sexes differently. Parents and offspring should also have no control over which patch type offspring develop in, and mating should take place among individuals coming from different patches. Its application to reptiles posits differential fitness for sexual phenotypes depending on a parameter correlated with their incubation temperature. Whereas these conditions indeed select for environmental sex determination in a theoretical model (Bull, 1981), they have never been conclusively demonstrated in reptiles. Though not yet validated, alternative models bring new perspective on this subject (Hulin & Guillon, 2007; Julliard, 2000; Reinhold, 1998; Roosenburg, 1996).

During extreme climatic events, greater numbers of unisex nests can be produced. If these conditions persist in the long term, the population sex ratio would become highly biased and could present an evolutionary drawback of TSD in turtles. This evolutionary question is today of great importance because of predicted rapid climate change and associated global warming (IPCC, 2001). To assess the evolutionary significance of TSD in turtles, we tried to answer three questions. First, we theoretically compared strategies producing unisex or mixed-sex ratio within a nest and their contributions to the population. Second, we looked for the risk of extinction of turtle populations according to the brood sex ratio strategy (mixed or unisex nests) used by individuals. Third, to compare our theoretical predictions with real-world scenarios we reviewed the literature and calculated the proportion of unisex nests in different turtle populations. Our results are relevant to the discussion on the evolution of TSD and its consequences on turtle populations.

## 11.2 UNISEX OR MIXED SEX RATIO STRATEGY IN A NEST: WHICH IS BEST?

Let us take first a simple model of population dynamics with constant population size  $N$  and constant average brood sex ratio in the population  $sr$  measured in male frequency. Two strategies will be examined: a unisex sex ratio strategy (USRS), where clutches produce all males with a frequency  $sr'$ , or all females with a frequency  $1 - sr'$ , and a mixed sex ratio strategy (MSRS) where the male frequency within a clutch is  $sr'$  and the female frequency is  $1 - sr'$ . During its lifetime, an individual will produce  $K$  eggs in  $L$  clutches. The brood size is then  $K/L$ . The distribution of sex ratios produced by this individual is obtained from a binomial distribution with the total number of events being the total number of clutches for USRS (because a clutch will be all male or all female) or the total number of eggs for MSRS (because an egg is either male or female). Note that these two strategies are the two extremes of a continuum. The distribution of sex ratios is then  $B(L, sr')$  for the individuals that use USRS and  $B(K, sr')$  for the individuals that use MSRS (Figure 11.1). Note that the total number of eggs in both cases is  $K$ , and therefore the strategy does not influence the global output of juveniles.



**FIGURE 11.1** Distribution of primary sex ratios produced during an individual's lifetime that uses unisex or mixed sex ratio strategy ( $sr' = 0.5$ ,  $N = 1000$ ,  $K = 100$ ,  $L = 10$ ). The contribution to the next generation measured in number of juveniles produced for each combination is shown in the lower row for  $sr = 0.3$ ,  $N = 1000$ ,  $K = 100$ , and  $L = 10$ .

The  $M$  male progeny of an individual will compete with other males of the population to reproduce, and the  $F$  females will compete with other females of the population. Thus, the contribution of an individual to the next generation will be  $\frac{M}{Ns_r}$  by the way of its male progeny and  $\frac{F}{N(1-s_r)}$  by the way of its female progeny (Shaw & Mohler, 1953).

The contribution of one individual using USRS who produces  $i$  unisex male clutches among the  $L$  clutches she produces during her lifetime is

$$\frac{iK}{Ns_rL} + \frac{(L-i)K}{N(1-s_r)L}$$

An individual using MSRS produces  $i$  male eggs among the  $K$  eggs she produces during her lifetime. The contribution of this individual to the next generation is therefore

$$\frac{i}{Ns_r} + \frac{K-i}{N(1-s_r)}$$

Then the average contribution  $C$  of strategy MSRS and USRS are estimated as the sum of the contribution of each brood composition weighted by its frequency among the possible broods,

$$C_{MSRS} = \sum_{i=0}^K \binom{i}{K} (sr')^i (1-sr')^{K-i} \left( \frac{i}{N sr'} + \frac{K-i}{N(1-sr')} \right)$$

As

$$\sum_{i=0}^x \binom{i}{x} (p)^i (1-p)^{x-i} i = E(B(x, p)) = x.p$$

and

$$\sum_{i=0}^x \binom{i}{x} (p)^i (1-p)^{x-i} (x-i) = E(B(x, 1-p)) = x.(1-p)$$

then

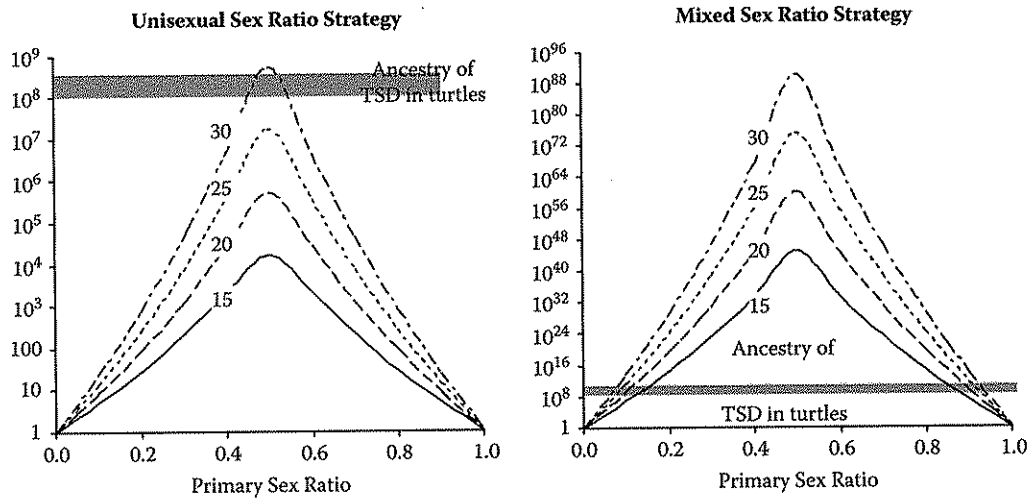
$$C_{MSRS} = C_{USRS} = \frac{K}{N} \left( \frac{sr'}{sr} + \frac{1-sr'}{1-sr} \right)$$

Overall, the choice of using a mixed or unisex sex ratio strategy has no influence on the contribution to the next generation and is therefore essentially neutral (Figure 11.1).

Whereas the use of mixed or unisex sex ratio strategy is not under selection, there is still one potential difference in the probability of extinction of the population when all individuals use USRS versus MSRS. If mortality occurs mainly at the level of the nest, such as egg destruction during incubation (i.e., a nest is destroyed or not, Eckrich & Owens, 1995; Girondot et al., 2002), each year very few nests may effectively contribute to the population. Under the USRS scenario, juveniles that survive for a particular year have a higher probability of being of the same sex. Consider the case when only one nest escapes destruction each year and adults reproduce  $Y$  years in this population. Then the probability that simply by chance the population becomes unisex is  $Ysr' + Y(1-sr')$ . The first occurrence of a unisex outcome for the population will follow a geometric distribution with parameter  $p = Ysr' + Y(1-sr')$  and with mean  $\frac{1}{p}$ .

Hence, for  $Y=20$  and  $sr'=0.5$  (i.e., half of the nests are all male producing and the other half are all female producing), a unisex outcome for the population will occur once every ~500,000 years, on average (Figure 11.2). Although this may appear to be a relatively rare event, one should recall that TSD is an ancient character in reptiles, having appeared between 100 and 300 million years ago (Janzen & Krenz, 2004). In the case of a MSRS, the probability that simply by chance the population becomes unisex is  $\frac{sr'^{YK}}{L} + \frac{(1-sr')^{YK}}{L}$ . For  $Y=20$ ,  $sr'=0.5$ , and  $K/L=10$ , a unisex outcome for the MSRS population is expected to occur once every  $\sim 10^{60}$  years, on average (Figure 11.2).

When mortality occurs at the scale of the whole nest, a lineage using the USRS should face a higher probability of extinction over the long term, so only those using the MSRS should have been able to survive until now. This type of group-selection argument is similar to explanations for the long-term advantage of sex (Gouyon et al., 1989; Nunney, 1989).



**FIGURE 11.2** Mean time in years to arrive at unisex populations in species with TSD when one nest per year escapes destruction. The number of cohorts contributing to reproduction is shown on the top of each curve. Note that the placement of the bar “Ancestry of TSD in turtles” (estimated time since TSD has appeared in turtles) is the same in both graphs although the scale is different. Mixed sex ratio strategy:  $K/L = 10$ .

### 11.3 REVIEW OF THE PROPORTION OF UNISEX NESTS FROM FIELD STUDIES

#### 11.3.1 SAMPLING REQUIREMENTS

Ideally, to assess the exact proportion of unisex nests in turtle species it would be necessary to classify the sex of all hatchlings in all nests deposited by each turtle of the considered population during its total lifespan—a logistical impossibility. Therefore, for our purposes we reviewed the published literature for estimates of offspring sex ratios in turtles. We consider the following components of study design as minimal requirements for adequately assessing the level of unisex nests in a turtle population.

##### 11.3.1.1 Fair Spatial Sampling

For turtle species with TSD, the sex ratio in a nest is dependent on the thermal conditions where the nest develops. As most nesting areas cannot be considered thermally homogeneous (Hays et al., 1995; Mrosovsky et al., 1984a), the spatial location of a nest has an impact on its hatchling sex ratio. An estimation of the proportion of unisex nests is then representative only of the part of the nesting area where nests have been sampled. For example, in sea turtle species it is known that females may nest on several beaches (Eckert et al., 1989); these beaches can be thermally heterogeneous (shade due to the vegetation, composition of the sand, cooling effect of the tide, and so on). Therefore, studies aiming to estimate the proportion of unisex nests at the scale of the nesting beach must sample nests in the different areas of the beach. In addition, studies aiming to estimate this proportion at the scale of a geographic area must sample nests in each nesting beach.

##### 11.3.1.2 Fair Temporal Sampling

Nest sex ratio is influenced by the seasonality of nesting. Thermal conditions vary at intra- and inter-annual scales, causing the nest sex ratios to vary during and between nesting seasons (Godfrey & Mrosovsky, 1999). Therefore, an estimation of the proportion of unisex nests can be biased if the field study is constrained either to a fraction of the entire nesting season or to a single nesting season.

### 11.3.1.3 Accurate Classification of Sex

The sex of turtle hatchlings can be determined by several methods. Due to a lack of external morphological differences between male and female hatchlings, direct observation of gonadal structure has been considered to be the most accurate method of classifying sex (Mrosovsky & Godfrey, 1995). However, direct observation usually requires the sacrifice of hatchlings being studied. As many turtle species are protected, some authors have used indirect estimates of nest sex ratio. These methods are based on models using temperature, or a proxy of temperature (e.g., duration of incubation), to estimate the sex ratio of the nest. However, because of differences between individuals in the consequences of thermal conditions on sex, these indirect methods are imprecise and must be interpreted with caution. The radioimmunoassay (RIA) of testosterone is another indirect method to classify the sex of hatchlings without killing them, but it must be parameterized for each species to which it is applied. To date, this method (Lance & Valenzuela, 1992) has been successfully used only for *Podocnemis expansa* (Valenzuela, 2001a; Valenzuela et al., 1997). An early report of the effectiveness of RIA in sexing loggerhead sea turtle hatchlings by Crain et al. (1995) has not been successfully replicated (Merchant-Larios, 1999).

The primary method then remains the direct observation of hatchlings' gonads. Most studies focused only on a small sample of hatchlings or eggs to limit the consequences for the population. When small samples are used to estimate the nest sex ratio, another potential bias could arise if the sample is not representative of all hatchlings within a clutch. Indeed, thermal conditions are known to vary within the nest causing male and female hatchlings to be more or less frequent depending on the position in the nest (Georges, 1992; Godfrey et al., 1997).

### 11.3.2 DATA FROM THE LITERATURE

For the purposes of our study, we estimated the proportion of unisex nests in different turtle populations based on data from studies where sexual phenotype was determined by the structure of the gonad or by RIA of testosterone, and sex ratio values are independently given for each natural nest. (Note that we excluded studies that focused on nests that had been manipulated, such as by relocation to a protected hatchery.) Thirty-three studies were retained using these criteria (Table 11.1). Twenty-two of these also give the exact number of hatchlings sexed for each nest. As a turtle population is difficult to delimit, especially in the case of sea turtles, in the present section the term "population" is used to designate geographically distinct nesting areas that may not always fit the theoretical concept in population biology.

From these 33 studies, we compiled data for 25 populations of 13 species (Table 11.1). The number of populations for each species varies from one (for seven species) to six (for *Caretta caretta*). For each population, we have data covering 1 (for 13 cases) to 6 years (for *Chrysemys picta* in Illinois and *Dermochelys coriacea* in French Guiana and Suriname), with data for at least two nesting seasons for other 11 populations. Some studies focused on small numbers of nests (Table 11.1). Three were conducted on only one nest and no other studies concerned the same species or the same population—Bull & Vogt (1979) on *Trionyx spiniferus*, Demuth (2001) on *Gopherus polyphemus*, and Dalrymple et al. (1985) on *Eretmochelys imbricata* in Florida. One population was represented by only three nests—Alho (1985) on *Podocnemis expansa* in Brazil—and one by only two nests—Kaska et al. (1998) on *Caretta caretta* in Cyprus). Six studies focused on one to five nests but concerned populations that were also studied in other years. We sought to include as much data as possible to facilitate our analyses without sacrificing the essential criteria set out previously.

As for all meta-analyses, we observed a large heterogeneity in the quality of data. For instance, inter-seasonal and intra-seasonal temporal variability is differentially described in all populations. For example, half of the studies (24 studies of 33) sampled nests at different days encompassing an important proportion of the nesting season (Table 11.1). Also, the spatial variability was different, depending on the study (Table 11.1): only six studies sampled nests in different nesting beaches/

**TABLE 11.1**  
**Description of the Studies Used for the Review\***

Species	Geographic Area	Year(s)	Temporal Sampling	Spatial Sampling	Sexual Phenotype Identification	Hatchlings per Nest	Nests
<i>Caretta caretta</i>	Cyprus	1995 <sup>5</sup>	10-Jun and 27-Jul	2 beaches (Akdeniz and Karpaz)	Eggs sampled at different depths in the nest a few days before emergence and artificially incubated thereafter	9-21	2
	Espirito Santo and Bahia (Brazil)	1996 <sup>6</sup> and 1997 <sup>6</sup> (data of both years mixed together)		2 beaches (Comboios and Praia do Forte)	Hatchlings sampled during emergence	10	51
	Cape Canaveral (Florida)	1986 <sup>3</sup>	Daily every 2 weeks from 17-May to 16-Aug		Hatchlings sampled during emergence	10	45
		1987 <sup>4</sup> and 1988 <sup>4</sup>	Daily every 2 weeks from 17-May to 16-Aug		Hatchlings sampled during emergence	10	76
	Natal (South Africa)	1984 <sup>2</sup> and 1985 <sup>2</sup>	Daily from 27-Oct-1984 to 01-Jan-1985		Eggs sampled in the nest at the end of the thermosensitive period and artificially incubated thereafter	20-25	17
	South Carolina	1979 <sup>1</sup> , 1980 <sup>1</sup> , and 1982 <sup>1</sup>		3 islands (Sand island, South island, and Kiawah island)	Hatchlings sampled during emergence	10	18
	Turkey	1995 <sup>5</sup>	Daily from 8-Jun to 2-Aug	4 beaches (Dalyan, Fethiye, Patara, and Kizilot)	Hatchlings found in the nest a few days after emergence or eggs sampled at different depths in the nest a few days before emergence and artificially incubated thereafter	9-21	6
		2000 <sup>7</sup> , 2001 <sup>7</sup> , and 2002 <sup>7</sup>	Daily between Jun and Jul		Hatchlings found in the nest a few days after emergence or eggs sampled at different depths in the nest a few days before emergence and artificially incubated	2-6	21
<i>Carettochelys insculpta</i>	Northern Territory (Australia)	1986 <sup>8</sup>	Daily between 6-Aug and 15-Dec		Hatchlings sampled in nests a few days before emergence	4-11	6

(Continued)

TABLE 11.1 (continued)  
Description of the Studies Used for the Review\*

Species	Geographic Area	Year(s)	Temporal Sampling	Spatial Sampling	Sexual Phenotype Identification	Hatchlings per Nest	Nests
<i>Chelonia mydas</i>	Ascension Island	1996 <sup>8</sup> , 1997 <sup>9</sup> , and 1998 <sup>9</sup>	11 days between 03-Jan and 08-Apr	3 beaches (South West beach, Long beach, and North East beach)	Eggs sampled in nests a few days before emergence and artificially incubated	—	140
		1999 <sup>13</sup>			Hatchlings sampled in the nest after emergence	3-9	20
	Caribbean coast (Costa Rica)	1980 <sup>11</sup>		Tortuguero beach divided into high-, mid-, and low-beach	Eggs sampled in the nest a few days before emergence and artificially incubated or hatchlings sampled during the emergence	20	15
	Cyprus	1995 <sup>5</sup>	5 days between 8-Jun and 25-Jul	2 beaches (Akdeniz and Karpaz)	Eggs sampled at different depths in the nest a few days before emergence and artificially incubated	9-21	5
		1998 <sup>12</sup>	Daily or every 2 days between May and Oct	2 beaches (Alagadi and west coast of the island)	Dead hatchlings found in the nest a few days after emergence	1-10	48
	Suriname	1980 <sup>10</sup>	Daily from 27-May to 9-Jun		Hatchlings sampled during emergence	10	12
		1981 <sup>15</sup> and 1982 <sup>15</sup>	Every 2-4 days from 7-Mar to 12-Aug		Hatchlings sampled during emergence	10	113
		1993 <sup>14</sup>	Nearly every day between 3-Mar and 1-Sep	Beach divided into vegetation area, vegetation line, and sand area	Hatchlings sampled during emergence	3-10	79
<i>Chelydra serpentina</i>	Mississippi River (Illinois)	1999 <sup>16</sup>			Hatchlings sampled during emergence	10	14
<i>Chrysemys picta</i>	Ontario (Canada)	1984 <sup>17</sup>			Hatchlings sampled in nests a few days before emergence	—	21

	1980 <sup>21</sup>	Nearly every day from 31-May to 28-Jun	Hatchlings sampled in nests a few days before emergence	—	2
	1989 <sup>18</sup> , 1990 <sup>18</sup> , 1991 <sup>18</sup> , and 1992 <sup>18</sup>	Almost daily in Jun	Hatchlings sampled in nests a few days before emergence	10	116
	1995 <sup>19</sup>	Almost daily in Jun	Living or dead hatchlings remaining in nests after emergence	6-14	10
	1995 <sup>20</sup>	Almost daily in Jun	Nesting area divided into low, intermediate, and high vegetation cover	> 6	157
<i>Dermaochelys coriacea</i>	1981 <sup>22</sup> , 1983 <sup>22</sup> , 1984 <sup>22</sup> , and 1985 <sup>22</sup>	Daily between 14-Jun and 31-Jul	Hatchlings remaining in nests after emergence	8-40	34
	1982 <sup>15</sup>	Every 2-4 days from 7-Mar to 12-Aug	Hatchlings sampled during emergence	10	29
	1993 <sup>14</sup>	Nearly every day between 3-Mar and 1-Sep	Hatchlings sampled during emergence	3-10	27
	1994 <sup>23</sup>	Beach divided into vegetation area, vegetation line, and sand area	Hatchlings sampled in nests laid below the high tide line	—	18

(continued)

TABLE 11.1 (continued)  
Description of the Studies Used for the Review\*

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Species	Geographic Area	Year(s)	Temporal Sampling	Spatial Sampling	Sexual Phenotype Identification	Hatchlings per Nest	Nests
<i>Eremochelys imbricata</i>	Soldier Key (Dade County, Florida)	1982 <sup>24</sup>	One nest		70 hatchlings among the 141 hatchlings of the nest (all hatchlings were dead)	70	1
	Buck Island	1994 <sup>25</sup>			Dead hatchlings found in the nest a few days after emergence	574 from 51 nests	51
<i>Graptemys</i> spp.	Mississippi River (Illinois)	1978 <sup>27</sup>			Hatchlings sampled in nests a few days before emergence	—	202
		1980 <sup>21</sup>	Nearly every day from 31-May to 28-Jun		Hatchlings sampled during emergence	—	75
		1982 <sup>26</sup>	Nearly every day from Jun to Sept		Hatchlings sampled in nests a few days before emergence	At least 8	14
<i>Gopherus polyphemus</i>	Cape Canaveral (Florida)	—28	One nest		Hatchlings sampled in the nest before emergence	5	1
<i>Malacochersus temminckii</i>	Apalachicola River (Florida)	1990 <sup>29</sup> and 1991 <sup>29</sup>			Hatchlings remaining in nests after emergence	6	47
<i>Podocnemis expansa</i>	Pará State (Brazil)	1984 <sup>30</sup>			Hatchling sampled during emergence	—	3
	Middle Caquetá River (Colombia)	1993 <sup>31</sup> and 1994 <sup>31</sup>		3 beaches (Yarumal, Centro, and Guadual)	RIA of testosterone for all living hatchlings sampled between hatching and emergence	19–42	6
		1997 <sup>32</sup> and 1998 <sup>32</sup>			RIA of testosterone for living hatchlings sampled between hatching and emergence	30	3

Species	Location	Year	Sampling Period	Sampling Method	Number of Hatchlings
<i>Podocnemis unifilis</i>	Guapore River (Rondonia, Brazil)	1990 <sup>33</sup>	Daily from Aug to Dec	Hatchlings remaining in nests after emergence	15
<i>Trionyx spiniferus</i>	Mississippi River (Illinois)	1978 <sup>21</sup>	Nearly every day from 31-May to 28-Jun	Hatchlings sampled in nests before emergence	At least 8

\* In the absence of notation, live hatchlings were sampled. The classification of hatchling was done by observation of the histological structure of the gonads unless otherwise noted. A cell with no data means that the information was not found in the corresponding article. A blank cell in the "Spatial sampling" column means that the study focused on only one beach/nesting area. Nesting beach/area is named in the "Spatial Sampling" column when listed in the source.

<sup>1</sup>Mrosovsky et al. (1984); <sup>2</sup>Maxwell et al. (1988); <sup>3</sup>Mrosovsky & Provancha (1989); <sup>4</sup>Mrosovsky & Provancha (1992); <sup>5</sup>Kaska et al. (1999); <sup>6</sup>Mrosovsky et al. (1999); <sup>7</sup>Kaska et al. (2006); <sup>8</sup>Georges (1992); <sup>9</sup>Doody et al. (2004); <sup>10</sup>Mrosovsky (1982); <sup>11</sup>Spotila et al. (1987); <sup>12</sup>Broderick et al. (2000); <sup>13</sup>Godfrey et al. (1996); <sup>14</sup>Godfrey et al. (2002); <sup>15</sup>Mrosovsky et al. (1984); <sup>16</sup>Kolbe & Janzen (2002); <sup>17</sup>Schwarzkopf & Brooks (1985); <sup>18</sup>Janzen (1994b); <sup>19</sup>Weisrock & Janzen (1999); <sup>20</sup>Valenzuela & Janzen (2001); <sup>21</sup>Vogt & Bull (1984); <sup>22</sup>Rimblot-Baly et al. (1987); <sup>23</sup>Binckley et al. (1998); <sup>24</sup>Dalrymple et al. (1985); <sup>25</sup>Wibbels et al. (1999); <sup>26</sup>Bull & Vogt (1979); <sup>27</sup>Bull (1985); <sup>28</sup>Demuth (2001); <sup>29</sup>Ewert (1994); <sup>30</sup>Alho et al. (1985); <sup>31</sup>Valenzuela et al. (1997); <sup>32</sup>Valenzuela (2001a); <sup>33</sup>De Souza & Vogt (1994).

areas and four sampled nests in different sub-areas of the beach (according to the surrounding vegetation or the position in the beach).

The collection methods used in selected studies to obtain the hatchlings also differ greatly. In the majority of the cases, authors sampled from 10 to 20 hatchlings per clutch during emergence. Some other studies sampled embryos in the nest a few days before the estimated end of incubation and let embryos complete incubation in the laboratory (Doody et al., 2004; Kaska et al., 1998; Maxwell et al., 1988; Spotila et al., 1987). Because sexual differentiation of hatchling sex is thermally sensitive during the second third of incubation, we assumed that altering thermal conditions during the end of incubation did not alter the natural sex ratio. These two sampling methods result in an unbiased estimate of the offspring sex ratio if sampling is representative of eggs or hatchlings from different positions in the nest. Except for Kaska et al. (1998), the precision about the sampling within the nest was not given in the article, although some authors stated that they randomly sampled from all emergent hatchlings. Finally, some studies used dead hatchlings found in the nest after emergence had been observed (Broderick et al., 2000; Kaska et al., 2006; Wibbels et al., 1999). Although avoiding the problem of sacrificing living hatchling, this method may not represent an unbiased sample of hatchlings. Indeed, it is possible that dead hatchlings remaining in the nest were not randomly distributed in the nest and thus had experienced a different thermal regime of incubation when compared to the hatched eggs. For example, the thermal conditions at the bottom of the nest tend to be cooler than at the top of the nest (Kaska et al., 1998).

### 11.3.3 ANALYSES OF THE SEX RATIO DATA

Overall, the proportion of unisex turtle nests was 0.65 and of these unisex nests, a proportion of 0.72 was all female. The proportion of unisex nests was also calculated independently for each population and year. A hierarchical model was built to test for the origin of heterogeneity (species, population, and year) in the proportion of unisex nests. For this test, *Trionyx spiniferus* was removed, as this species possesses GSD. Data were divided into groups of unisex male, unisex female, and mixed sex nests. The likelihood of the observations was calculated using a multinomial model. In short, the  $-\ln$  likelihood of an observation of  $m_i$  unisex male,  $f_i$  unisex female, and  $u_i$  mixed sex ratio nests was calculated as

$$L_i = -\ln \left( \frac{(m_i + f_i + u_i)!}{m_i! f_i! u_i!} p m_i^{m_i} p f_i^{f_i} p u_i^{u_i} \right)$$

where  $p m_i$ ,  $p f_i$ , and  $p u_i$  respectively are the expected proportion of males, females, and mixed sex ratio nests. These expected proportions were calculated taking into account a species, *species + population* or *species + population + year* effect. The various likelihoods were compared using likelihood ratio test where  $-2(L_A - L_B)$  was distributed as a  $\chi^2$  with a degree of freedom equal to the difference in the number of parameters between model A and B. We found a highly significant species effect (LRT = 280.46, 22 df,  $p = 10^{-46}$ ), a population within species effect (LRT = 224.18, 44 df,  $p = 10^{-35}$ ), and a year within population and species effect (LRT = 275.62, 58 df,  $p = 10^{-30}$ ). Similar results are found if nests were grouped in unisex or mixed sex ratio nests; there was a highly significant species effect (LRT = 161.47, 11 df,  $p = 10^{-29}$ ), a population within species effect (LRT = 144.53, 11 df,  $p = 10^{-25}$ ), and a year within population and species effect (LRT = 95.63, 29 df,  $p = 10^{-9}$ ). These effects were significant whether including the population where only one nest was sampled or not.

Indeed, the proportion of unisex nests differed greatly between species, between populations, and between years (Table 11.2; Figure 11.3). For example, for *Caretta caretta* the proportion of unisex nests varied from 0.07 in Turkey to 0.73 in Brazil, and just within Turkey the proportion varied

**TABLE 11.2**  
**Proportion of Unisex Nests for Different Populations\***

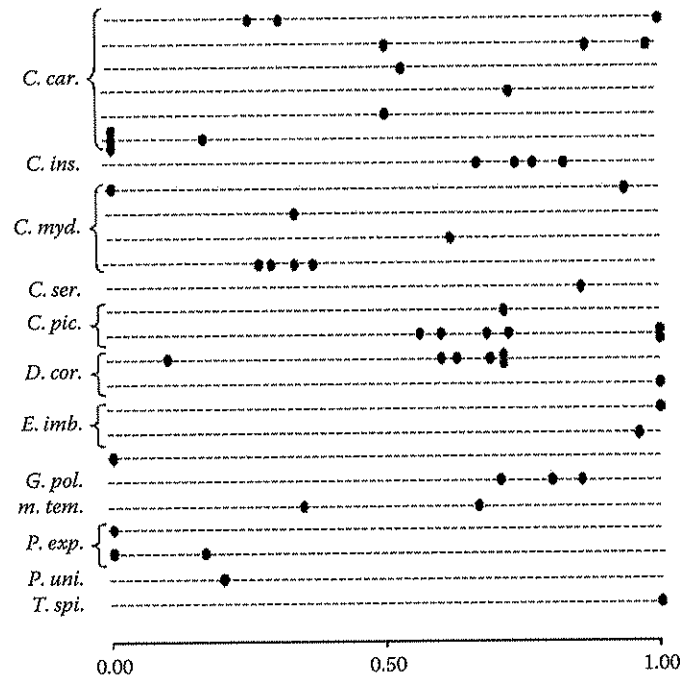
	Population	Prop. of Unisex Nests	No. of Nests
<i>Caretta caretta</i>	South Carolina	0.33	18
	Cape Canaveral (Florida)	0.82	121
	Natal (South Africa)	0.53	17
	Espirito Santo and Bahia (Brazil)	0.73	51
	Turkey	0.04	27
	Cyprus	0.50	2
<i>Carettochelys insculpta</i>	Northern Territory (Australia)	0.78	146
<i>Chelonia mydas</i>	Cyprus	0.85	52
	Caribbean coast (Costa Rica)	0.33	15
	Ascension Island	0.62	21
	Suriname	0.31	204
<i>Chelydra serpentina</i>	Mississippi River (Illinois)	0.86	14
<i>Chrysemys picta</i>	Wolf Howl Pond (Canada)	0.71	21
	Mississippi River (Illinois)	0.67	129
<i>Dermodochelys coriacea</i>	Suriname	0.59	90
	Pacific coast (Costa Rica)	1.00	18
<i>Eretmodochelys imbricata</i>	Soldier Key (Florida)	1.00	1
	Buck Island	0.96	51
<i>Gopherus polyphemus</i>	Cape Canaveral (Florida)	0.00	1
<i>Graptemys</i> spp.	Mississippi River (Illinois)	0.78	291
<i>Malacochersus temminckii</i>	Apalachicola River (Florida)	0.51	47
<i>Podocnemis expansa</i>	Pará State (Brazil)	0.00	3
	Tamanco (Colombia)	0.11	9
<i>Podocnemis unifilis</i>	Guapore River (Brazil)	0.20	15
<i>Trionyx spiniferus</i>	Mississippi River (Illinois)	0.00	1

\* Note that *Trionyx spiniferus* is a species with GSD.

from 0.00 in 2000, 2001, and 2002 to 0.17 in 1995. The variability of the proportion of unisex nests reported in the literature is in accordance with the theoretical prediction of our model. Indeed, we predicted that unisex and mixed sex strategies are evolutionarily neutral.

Most studies based their estimates of nest sex ratios on a sample of hatchlings. If the sampled nest was mixed, the probability of observing a unisex sample of hatchlings is inversely proportional to the number of hatchlings sampled. Except in the rare cases when all hatchlings from a nest were sexed, most of the studies had relatively small sample sizes and thus it was generally not possible to reject at the 5% significance level the hypothesis that a nest was actually mixed when the observed sample showed only one sex. Therefore, the proportion of mixed nests reported in the literature may be underestimated.

The overall sex ratio is biased toward females (66%) but mean sex ratios in nests were also calculated independently for each population (Table 11.3) and year (not shown). A hierarchical analysis (see previous paragraph) showed that there are significant differences between species (LRT = 330.51, 10 df,  $p = 10^{-65}$ ), populations of the same species (LRT = 728.44, 17 df,  $p = 10^{-153}$ ) and years within a population (LRT = 875.50, 37 df,  $p = 10^{-172}$ ). Only a few populations showed a higher proportion of males, and the single population where only males were reported in the nest is



**FIGURE 11.3** Proportion of unisex nests for all sampled populations of all species. Each line represents a population. Each square dot on the line represents the proportion of unisex nests for a given year. Note that *Trionyx spinifera* is a species with GSD.

atypical (Dalrymple et al., 1985). More generally, studies where nest sex ratios are skewed relative to 0.5 are also those studies with the lowest number of sampled nests. Nest sex ratios can be considered as biased toward females because 18 populations (including studies where the number of studied nests was high) out of 22 showed a female biased mean sex ratio. This strong bias of sex ratios toward females is at the limit of evolutionary possibility (Charnov & Dawson, 1989) and may suggest that primary sex ratio of some populations is not at the evolutionary equilibrium. One possible cause of this general bias toward females in populations of all species could be global warming.

#### 11.4 GLOBAL WARMING AND THE FATE OF TSD TURTLES

Here we focus on threats specific to reptile species with TSD, leaving aside more general threats concerning cold-blooded vertebrates, temperate biodiversity, or aquatic animals. Present scenarios of greenhouse gas emissions predict a doubling of atmospheric CO<sub>2</sub> to be reached between years 2050 and 2100 (IPCC, 2001). According to simulations, this should be followed by a 2 to 11°C increase in global temperatures (Stainforth et al., 2005). This warming is expected to have a profound impact on the offspring sex ratio of turtle populations, as higher temperatures could impede the production of males (Janzen, 1994a). Reptiles with TSD have already been confronted to major climatic deteriorations (Rage, 1998) and yet have survived until now, although present warming is believed to be much more sudden than past episodes. A rapid evolutionary response would be necessary for species to “catch up” to the speed of climate change. For an appropriate response to occur, there must be either heritable behavioral/physiological variation among individuals in a population or the adaptive plasticity of a phenotypic trait. The frequency-dependent selection for the production of males could then result in various outcomes, as follows.

**TABLE 11.3**  
**Mean Sex Ratios (percentage of males) and Variance among Nests and Number of Studied Nests of Populations\***

	Population	Sex Ratio	Variance	No. of Nests
<i>Caretta caretta</i>	South Carolina	0.47	0.007	18
	Cape Canaveral (Florida)	0.09	0.0004	121
	Natal (South Africa)	0.64	0.011	17
	Espirito Santo and Bahia (Brazil)	0.34	0.088	51
	Turkey	0.33	0.002	27
	Cyprus	0.09	0.007	2
<i>Carettochelys insculpta</i>	Northern Territory (Australia)	0.30	0.028	6
<i>Chelonia mydas</i>	Cyprus	0.04	0.002	52
	Caribbean coast (Costa Rica)	0.57	0.009	15
	Ascension Island	0.19	0.004	21
	Suriname	0.34	0.001	204
<i>Chelydra serpentina</i>	Mississippi River (Illinois)	0.09	0.005	14
<i>Chrysemys picta</i>	Wolf Howl Pond (Canada)	0.13	0.002	21
	Mississippi River (Illinois)	0.46	0.001	129
<i>Dermochelys coriacea</i>	Suriname	0.38	0.003	90
	Pacific coast (Costa Rica)	0.00	0	18
<i>Eretmochelys imbricata</i>	Soldier Key (Florida)	1.00		1
<i>Gopherus polyphemus</i>	Cape Canaveral (Florida)	0.20		1
<i>Graptemys</i> spp.	Mississippi River (Illinois)	0.40	0.001	291
<i>Malacochersus temminckii</i>	Apalachicola River (Florida)	0.34	0.003	47
<i>Podocnemis expansa</i>	Pará State (Brazil)	0.03	0.001	3
	Tamanco (Colombia)	0.25	0.011	9
<i>Podocnemis unifilis</i>	Guapore River (Brazil)	0.58	0.007	15
<i>Trionyx spiniferus</i>	Mississippi River (Illinois)	0.54		1

\* Note that *Trionyx spiniferus* is a species with GSD.

First, TSD could be lost in favor of GSD, thereby ensuring an equilibrated primary sex ratio. Although this transition probably occurred in the past of six turtle lineages (Janzen & Krenz, 2004), it is unlikely that many turtle species could respond in this way in a short time. The transition from TSD to GSD would imply that genetic variation for sex determination already includes individuals with GSD instead of TSD, or that TSD may mutate to GSD in the next generations. To date, only one lizard species is reported to exhibit both GSD and TSD (Shine et al., 2002); this has not been discovered in any turtle species so far.

Second, TSD could evolve by a displacement of the pivotal temperature (toward a higher value) or an extension of the transitional range of temperature (a flattening of the response to temperature, especially in higher temperatures). This mechanism would allow the production of males in the future at temperatures that are now completely feminizing. There is ample evidence for the existence of clutch effects on the sex ratio of laboratory-incubated eggs, which may be the expression of

genetic or maternal effects (Ewert et al., 1994; Mrosovsky, 1988). Assuming the absence of maternal effects, two studies have found high heritable genetic variation for sex ratio in a population of map turtles (*Graptemys ouachitensis*:  $h^2 = 0.82$ ; Bull et al., 1982a) and in a population of snapping turtles (*Chelydra serpentina*:  $h^2 = 0.34$  to  $0.76$ ; Janzen, 1992). This indicates the potential for evolutionary change in response to sex ratio selection in TSD turtles. A simulation model of sex ratio evolution in *Chrysemys picta* in response to climate change parameters also favored this scenario (Morjan, 2003b).

However, environmental variability could override the expression of individual variation and thus hamper the selection on sex ratio. In the case of TSD, individual variation could be overridden if eggs in natural nests were incubated under conditions that would always give males or always give females, regardless of genetic factors. Then the reaction norm to temperature during sex determination would have little influence on the sex that is finally expressed. The effective heritability of the reaction norm could thus be very low (Bull et al., 1982a). Comparing the response to temperature between populations has revealed no consistent trend of higher pivotal temperatures in warmer climate for turtles (Bull et al., 1982a; Ewert et al., 2004; Ewert et al., 1994, 2005; Mrosovsky, 1988; Vogt & Flores-Villela, 1992) and for a TSD lizard (Doody et al., 2006). Geographic variation in nest-site choice compensating for temperature variation seems to explain this lack of a trend (Doody et al., 2006; Ewert et al., 1994, 2005; Morjan, 2003b). This has led to the proposal that adaptation to increasing temperature could evolve more easily by the evolution of nest site choice (Bulmer & Bull, 1982; Janzen & Morjan, 2001). The presence of other maternal effects, such as the possible influence of yolk hormones (Bowden et al., 2000), could also prevent the action of selection on genetic variation for the sex ratio (St. Juliana et al., 2004).

Third, as lower temperatures are usually masculinizing in turtles, females could select cooler places for nesting. Micro-environmental heterogeneity of temperatures is well documented on nesting grounds—nests under vegetation or closer to water are usually cooler, giving the females the opportunity to select a specific temperature regime for their brood (Janzen, 1994b; Kamel & Mrosovsky, 2005; Morjan, 2003b; Mrosovsky et al., 1986<sup>a</sup>). As noted previously, it has been observed that female turtles tend to nest in shadowed places in warmer climates (Ewert et al., 1994, 2005; Morjan, 2003b). Bulmer and Bull (1982) suggested that if nesting behavior is heritable, nest temperature could change faster than thermal response of offspring sex. Indeed, repeatability of nest site choice by individual females has been found in marine turtles (Kamel & Mrosovsky, 2004, 2005) and freshwater turtles (Janzen & Morjan, 2001). Repeatability is a prerequisite for heritability but is not enough to conclude that the nesting behavior is heritable. Furthermore, individual preferences could be overridden if micro-environmental variation of temperatures between nests was of minor importance compared to climatic variation of temperatures (within the nesting season or across years). This would result in low repeatability in nest sex ratios (Valenzuela & Janzen, 2001) and low effective heritability for nest temperatures (Morjan, 2003a). Without heritability, nest site selection could still evolve as a plastic trait. According to this hypothesis, female turtles would modify the placement of their nest depending on the thermal environment encountered in any given season. For this to occur, it would imply that females use environmental cues at the time of nesting that are correlated with temperatures during the thermosensitive period of incubation. This eventuality has not been properly evaluated, but hatchling sex ratios are often reported to be variable among years at the level of a population (Godfrey et al., 1996; Janzen, 1994b). This suggests that potential plasticity of the nesting behavior—including nest-site choice, nesting phenology, and nest depth—cannot fully compensate for climatic inter-annual variations. Alternatively, it has been proposed that the nest site may be inherited by imprinting (Julliard, 2000; Reinhold, 1998), i.e., nesting females would tend to return to the place or environment where they incubated and hatched themselves. As a result, the nest site would behave as a female-transmitted trait, which could constrain sex ratio evolution and possibly hinder the production of males (Freedberg & Wade, 2001).

Species could also evolve to nest at higher latitudes to compensate for the climatic change. This possibility is restricted to species that are able to undertake long-distance migrations,

principally sea turtles. Over evolutionary time, it is likely that this mechanism has provided new nesting beaches for refuge—during ice-age episodes, for example. Sea turtles are known to exhibit fidelity to nesting beaches or groups of nesting beaches (Bjorndal et al., 1985; Carr & Carr, 1972; Gyuris & Limpus, 1988; Mortimer & Portier, 1989; Schulz, 1975) but imperfect homing may allow shifts between nesting areas and occasional colonization events (Bowen et al., 1989; Meylan et al., 1990). However, it is difficult to assess whether this change could occur fast enough in response to global warming, and the increasing human development of shorelines for recreational, urban, or industrial activities could be an obstacle to the colonization of new nesting areas by sea turtles.

Fourth, breeding and nesting phenology could evolve to compensate for global warming. Seasonal shifts in the sex ratio from male-biased to female-biased have been reported in field studies (Doody et al., 2004; Maxwell et al., 1988; Rimblot-Baly et al., 1987). Nesting earlier (or later) in the season could ensure then that incubation takes place during a time when relatively cool temperatures still allow the production of males. It is known that turtle populations from different latitudes usually differ in the date of onset of their reproductive activity (Ewert et al., 2005). There is currently no evidence for heritable variability in nesting phenology. However, plastic behavioral response could occur via heat accumulation, as suggested for temperate freshwater turtles (Bull et al., 1982b; Congdon et al., 1983; Doody et al., 2004; Iverson et al., 1997; Obbard & Brooks, 1987) and loggerhead sea turtles (Weishampel et al., 2003).

Fifth, female turtles could modify their behavior by digging deeper (cooler) nests (Glen & Mrosovsky, 2004; Valenzuela, 2001b; Wilhoft et al., 1983). Individual variation has here been reported but is supposed to result mostly from differences in body size, with bigger (older) females digging deeper nests (Iverson et al., 1997; Morjan, 2003b; Vogt & Bull, 1982). However, in *Chrysemys picta* it seems that nest depth varies between populations, independent of female size (Morjan, 2003b). No study has yet addressed if there is size-independent variation of nest depth among individuals within a population or if females can modify this behavior in response to climatic fluctuations. In any case, the size of the turtle puts a limit on the depth of the nest, so that nests could go beyond that limit only by selection for a bigger size.

## 11.5 UNISEX VERSUS MIXED BROODS AND THE EVOLUTION OF TSD

The question of whether brood sex ratios are mixed or unisex in natural nests is relevant to the response of TSD to climate change. Natural selection can operate on a trait depending on its level of variation and heritability in a population. Among the main traits of TSD that show or may show heritability in TSD reptiles are the sex determination of embryos by temperature and the choice of nesting site. What does the proportion of mixed sex broods in natural nests tell us about individual variation? There are various possible reasons for mixed offspring sex ratios. The thermal environment may be variable within the nest, ranging from masculinizing to feminizing temperatures. The sex of the embryo would then depend on its position within the nest. The larger the clutch, the bigger the nest chamber, so thermal conditions may differ from the bottom (usually cooler) to the top (usually warmer) and with higher diel variation of temperatures (Georges, 1992; Hanson et al., 1998; Wilhoft et al., 1983). In large clutches of sea turtles, metabolically generated heat of developing eggs is also responsible for higher temperatures in the core of the nest than at the side (Broderick et al., 2001; Godfrey et al., 1997; Maxwell et al., 1988). The heterogeneity of temperatures in the nest may thus favor the production of mixed sexes.

Alternatively, if temperatures do not vary much with the position in the nest, a mixed sex ratio may be the expression of genetic variation between embryos (Girondot et al., 1994). Indeed, each offspring inherits only one allele from its mother and one allele from its father, with multiple paternity further increasing the genetic diversity of the clutch (Pearse & Avise, 2001). At intermediate temperatures, genetic variation would result in different sexes being expressed by embryos developing at the same temperature. A mixed brood may also be expected if sex determination is not a deterministic process but rather a probabilistic process: any embryo would have a probability  $p(T)$ ,

$x$ ) of becoming male or female, depending on the temperature ( $T$ ) and genetic factors ( $x$ ). Under the latter hypothesis, mixed sex ratios could arise without temperature or even genetic variation but genetic factors would modify the probability of being a male or a female at a given temperature.

In any case, mixed sex ratios favor the heritability of sex determination. For individual variation in TSD to be effectively inherited, the variation must be expressed by embryos during sex determination. In the first case, when nest conditions vary from the masculinizing to feminizing temperatures, some proportion of the embryos must develop under intermediate temperatures that allow the expression of genetic variation. In the second case, when temperature does not vary within the nest, the observation of mixed sex ratios is direct or indirect evidence for the expression of genetic factors: if sex determination is a deterministic process, then mixed sex ratios can only result from the expression of genetic variation; if sex determination is a probabilistic process, then mixed sex ratios indicate either a temperature where any small genetic variation would have a high influence on sex ratios—i.e.,  $T$  such that  $0 < p(T, x) < p(T, x+\delta) < 1$ —or the expression of high genetic variation within the clutch—i.e.,  $(x_i, x_j)$  such that  $p(T, x_i) = 0$  and  $p(T, x_j) = 1$ . Similarly, the fact that all clutches do not produce only females or only males at a given time favors the hypothesis that nest site choice has an influence on sex ratio, and could be effectively heritable.

Overall, prospects for TSD turtles may depend on the proportion of mixed and unisex broods detected in populations. Where unisex broods predominate, the thermal environment may severely constrain the evolution of sex ratios, with potentially grim consequences on the future of the population. This is especially true in populations already showing a large excess of all-female nests, an excess that is expected to increase as global climate continues to warm (e.g., *Caretta caretta* in Florida). Where mixed broods predominate (e.g., *Caretta caretta* in Turkey or *Chelonia mydas* in Suriname), traits like sex determination or nest site selection are more likely to evolve to equilibrate the sex ratio. Yet maternal effects may complicate the picture and make the future less predictable. In addition, the rapidity of temperature rise together with other human-related threats (poaching, incidental capture in fisheries, pollution, habitat loss) challenge the quiet rhythm of natural selection in long-lived turtles.

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