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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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## 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).

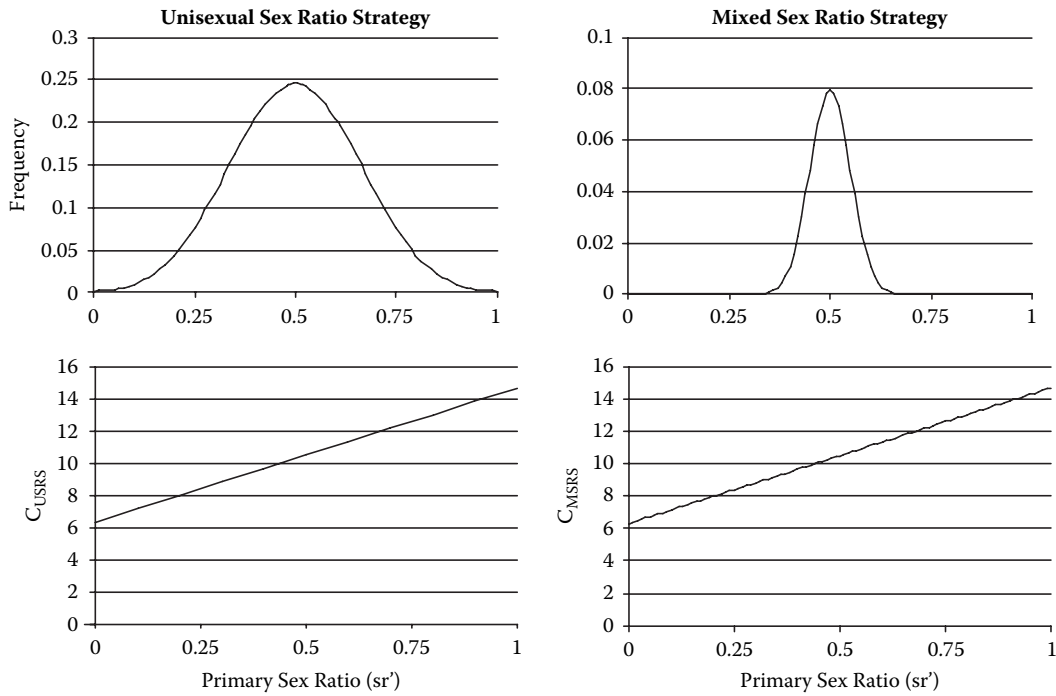
The precise timing when sex determination is sensitive to temperature during development has been studied in various reptiles. The timing is always linked with the first stages of gonadal development until the end of the second third of development (Pieau & Dorizzi, 2004). This homogeneity among various 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  $\frac{M}{Nsr}$  by the way of its male progeny and  $\frac{F}{N(1-sr)}$  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

$$\frac{iK}{NsrL} + \frac{(L-i)K}{N(1-sr)L}$$

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

$$\frac{i}{Nsr} + \frac{K-i}{N(1-sr)}$$

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,

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

As

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

and

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

then

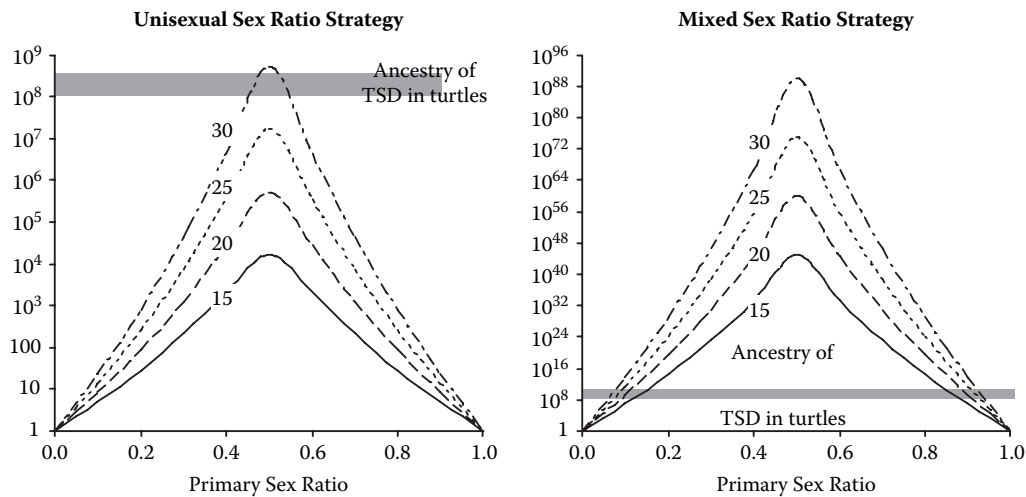
$$C_{MSRS} = C_{USRS} = \frac{K}{N} \left( \frac{sr'}{sr} + \frac{1-sr'}{1-sr} \right)$$

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  $sr'^Y + (1-sr')^Y$ . The first occurrence of a unisex outcome for the population will follow a geometric distribution with parameter  $p = sr'^Y + (1-sr')^Y$  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  $sr'^{Y.K/L} + (1-sr')^{Y.K/L}$ . For  $Y = 20$ ,  $sr' = 0.5$ , and  $K/L = 10$ , a unisex outcome for the MSRS population is expected to occur once every ~10<sup>60</sup> 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>Carettocheilus 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>9</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































