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Temperature-dependent sex determination and global change: are some species at greater risk?

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Abstract In species with temperature-dependent sex determination (TSD), global climate change may result in a strong sex ratio bias that could lead to extinction. The relationship between sex ratio and egg incubation at constant temperature in TSD species is characterized by two parameters: the pivotal temperature (*P*) and the transitional range of temperature that produces both sexes (TRT). Here, we show that the proportion of nests producing both sexes is positively correlated to the width of the TRT by a correlative approach from sex ratio data collected in the literature and by simulations of TSD using a mechanistic model. From our analyses, we predict that species with a larger TRT should be more likely to evolve in response to new thermal conditions, thus putting them at lower risk to global change.

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Introduction

The predicted rapid climate change in the next century (IPCC 2007) raises many questions about the possible impact of global warming on natural ecosystems. In this context, potential extinction and/or adaptation of species to new environmental conditions are of particular interest (Bell and Collins 2008). Apart from shifting their range, species can cope with global change in two principal manners: through phenotypic plasticity and microevolution. Phenotypic plasticity, when present, could immediately compensate for a moderate change in environmental conditions (Jump and Penuelas 2005). Indeed, the annual phenology of many species has changed in the past few years in response to modified environmental conditions (Parmesan 2006; Pertoldi and Bach 2007; Walther et al. 2002). In contrast, as microevolutionary responses depend on heritable genetic variation, it is unknown whether, and how fast, such changes can occur in the future.

Environmental sex determination (ESD) is a case of phenotypic plasticity in which sex is determined after conception according to the immediate environment (Bull 1983). This special case of phenotypic plasticity may not be advantageous and could potentially increase the risk of extinction under climate change. Indeed, in species showing ESD, rapid changes of environmental conditions might lead to large biases in offspring sex ratio, a critical life history trait. Therefore, ESD species are assumed to be highly sensitive to global climate change (Walther et al. 2002).

A particular form of ESD is called temperature-dependent sex determination (TSD). In TSD species, the sex of the embryo is determined by developmental temperature during a precise window of embryogenesis (Bull 1980; Raynaud and Pieau 1985). Among vertebrates, TSD is present in some fishes and common in reptiles (Valenzuela 2004). In oviparous reptiles, three patterns of TSD have been described according to the sex ratios produced as a function of constant incubation temperatures (Bull 1983; Ewert et al. 1994; Lang and Andrews 1994; Viets et al. 1994). In the TSD Ia pattern, present in turtles, low temperatures produce males and high temperatures produce females. The opposite occurs in some lizards (TSD Ib pattern). In the TSD II pattern, present in crocodile species, some turtles and some lizards, females are produced at low and high temperatures and males are produced at intermediate temperatures. Two parameters are commonly used to characterize these reaction norms (i.e., the sex ratios produced under constant incubation temperatures): (1) the pivotal temperature (*P*), i.e., the constant temperature at which both sexes are produced in equal proportions (sex ratio = 1:1), and (2) the transitional range of temperatures (TRT), i.e., the range of constant temperatures that yields both sexes in variable proportions. Note that there are two different values of *P* and TRT when considering the TSD II pattern. Hereafter, we will focus on the TSD Ia pattern, which is more frequently found in turtles.

TSD patterns and their associated *P* and TRT values are inferred from artificial incubation of eggs at constant temperatures at the laboratory. Under field conditions, where temperature fluctuates within the nest during the whole incubation period, *P* is sometimes used to estimate the sex ratio of natural nests (Mrosovsky et al. 1992). The value of *P* is an index of the temperature where there is a switch from a majority of one sex produced to the other. An indirect method of estimating sex ratios in natural nests from *P* is thus based on the proportion of the temperature-sensitive period of incubation during which the temperature is above *P* (reviewed in Georges et al. 2004). However, it is less clear how TRT relates to sex ratios in the field.

It is expected that global warming will result in increased atmospheric temperatures in a unusually short time scale (Stainforth et al. 2005). These new thermal conditions may modify several life history traits in natural populations of TSD species. Indeed, a multi-year study of a population of painted turtles (*Chrysemys picta*) reported that sex ratios were correlated with air temperature during the thermosensitive period of incubation (Janzen 1994). Increasing temperatures could thus prevent the production of the sex produced under low temperatures and push the population towards extinction if most offspring are of the same sex (Girondot et al. 2004; Hulin et al. 2008; Miller et al. 2004). Conversely, in response to global warming, natural selection could change aspects of the sex determination mechanisms or of maternal nesting behaviour of TSD species to assure production of both sexes under higher temperatures (Doody et al. 2006).

For TSD to evolve in response to selection requires the following: (1) variability in the response of sex determination of embryos to incubation temperature in natural populations, and (2) a heritable component of this variability. In the case of TSD Ia, under constant intermediate temperatures, identical thermal conditions can lead to different sexes for offspring from different nests (Ewert et al. 2005) or within the same nest. In other words, the response of sex determination to intermediate temperatures of incubation is variable between individuals. As an explanation, Bull et al. (1982a) argued that "there probably are genetic dispositions toward male or female determination within this range of intermediate temperatures". Experimental results have shown among-family variation for TSD (Bull et al. 1982a; Janzen 1992; Rhen and Lang 1998), but a significant portion of this variation could be due to non-genetic maternal effects (Bowden et al. 2000) or non-additive genetic effects. What proportion of sex ratio variation is due to heritable additive genetic effects remains unknown.

Another condition for the evolution of TSD is that heritable genetic variation should be expressed in the wild, i.e., environmental conditions should not totally override genetic factors in natural nests (Bull et al. 1982a). Eggs should experience environmental conditions such that genetic factors would be able to influence sexual differentiation. It is at intermediate temperatures that such genetic variation is expected to be expressed, because at extremely high or low values the temperature effect should override any genetic component. Indeed, the majority of unisexual nests is found at extreme incubation temperature (Bull 1985; Maxwell et al. 1988) where the genetic variation cannot be expressed. On the contrary, it has been argued that mixed nests experience intermediate incubation conditions that favor the expression of genetic variation among embryos (Hulin et al. 2008). Therefore, any study of the potential consequences of global warming on TSD species with respect to *P* and TRT values should take into account the proportion of mixed nests in study populations.

To assess the consequences of global warming on TSD species, we focused on the evolutionary potential of TSD reaction norms in relation to *P* and TRT. We chose turtle populations because of the interesting characteristics of this taxon. Indeed, the majority of TSD turtle species are reported to exhibit pattern TSD Ia (Ewert et al. 2004a), and many are species of conservation concern. We mined the literature to collect sex ratio data from turtle species, including data from incubation of eggs at constant temperatures to estimate the TSD reaction norm parameters (i.e., P and TRT), and from natural nests to estimate the proportion of nests with offspring of both sexes (i.e., mixed nests). We tested the relationship between the value of TRT and the proportion of mixed sex ratios with two complementary methods: (1) a correlative approach, and (2) simulations of a new mechanistic model of TSD. Finally, we discuss the potential of TSD turtles to adapt to global warming.

Materials and methods

Sex ratio data under constant incubation temperatures

We collected published data from TSD turtle populations for which sex ratios (here defined as the proportion of females), derived from incubation of eggs under diverse constant temperatures in the laboratory, were available (Table 1). Given the difficulty in clearly delimiting a turtle population, we use the term “population” to designate geographically distinct nesting areas that may not always fit the theoretical concepts of population biology.

TSD reaction norm

To describe the reaction norm (i.e., the sex ratios produced under constant incubation temperatures) for studied populations, we used two mathematical models developed by Girondot (1999) and Godfrey et al. (2003), and one new model. All models are based on equations with sigmoid shapes. These models differ only in the asymmetry around the inflexion point of the sigmoid curves.

In the first model (named No_{K}), no asymmetry is assumed. The relation between sex ratio and incubation temperature is calculated by a classic logistic equation:

$$sr(t) = \frac{1}{1 + e^{\frac{(P-t)}{S}}}, \qquad (1)$$

where t is the incubation temperature, P is the pivotal temperature, and S defines the shape of the transition from masculinizing to feminizing temperature. For a more precise description of parameters, see Godfrey et al. (2003).

In the second model (named K_{fix}), we assumed a potential asymmetry around the inflexion point of the sigmoid. In this model, the asymptote for the bottom or upper part of the curve can be more or less sharp, depending on the value of K_{fix}:

$$sr(t) = \left[\frac{1}{1 + (2^{e^{K}} - 1)e^{\frac{(P-t)}{S}}}\right]^{\frac{1}{e^{K}}}, \qquad (2)$$

where K determines the asymmetry of the function. If $K = 0$, Eq. 2 reduces to Eq. 1.

Because K is considered as constant for all t values in Eq. 2, the asymmetry values of the shape at lower and higher bounds of TRT are linked together (i.e., sharp at bottom implies smooth at top and vice versa, but not sharp at both or smooth at both). To counter this possible limitation, we developed a third model (named K_{var}) with K varying linearly with the temperature t:

$$sr(t) = \left[\frac{1}{1 + \left(2^{e^{K_1 \cdot t + K_2}} - 1\right)e^{\frac{(P-t)}{S}}}\right]^{\frac{1}{e^{K_1 \cdot t + K_2}}}, \qquad (3)$$

where K_1 and K_2 are the parameters describing the asymmetry, and are dependent on t.

We used the same method for model selection as in Godfrey et al. (2003), based on the corrected Akaike’s information criterion or AICc (Akaike 1974; Hurvich and Tsai 1989; Sugiura 1978). In all selected models, we calculated the two main parameters (P and TRT) describing the TSD reaction norm. P was estimated directly from Eqs 1, 2 and 3. To determine TRT precisely, we calculated the range of temperatures that produces sex ratios between 0.05 and 0.95 (Girondot 1999). For No_{K} and K_{fix} models (1) and (2), we used the relations in Eqs. 4, 5 and 6, proposed by Godfrey et al. (2003):

$$\mathrm{TRT} = 2 \cdot S \cdot \ln\left(\frac{0.05}{0.95}\right) \quad \text{for } No_{K}, \qquad (4)$$

$$\mathrm{TRT} = S \cdot \ln\left(\frac{0.05^{-e^{K}} - 1}{0.95^{-e^{K}} - 1}\right) \quad \text{if } K \leq 3 \quad \text{for } K_{\mathrm{fix}}, \qquad (5)$$

$$\mathrm{TRT} = S \cdot e^{K} \cdot \ln\left(\frac{0.95}{0.05}\right) \quad \text{if } K > 3 \quad \text{to avoid computing overflow, for } K_{\mathrm{fix}}. \qquad (6)$$

Considering the complexity of the K_{var} model, we obtained the TRT value by numerically approximating incubation temperatures producing sex ratios of 0.05 and 0.95. Standard errors were estimated by inverting the Hessian matrix of second order derivatives of likelihood for each couple of parameters.

Proportion of mixed nests under natural conditions

Here, we use ‘mixed nests’ to designate nests where authors found both sexes in their sample. Hulin et al. (2008) compiled observed offspring sex ratio data for several turtle populations and estimated the associated proportion of mixed nests. In the present study, we used data from selected populations for which we could also describe the TSD reaction norm (see previous section;

Table 1 Turtle populations for which data are available from the literature to describe the relationship between constant incubation temperature and sex ratio (*P* and TRT columns), and the proportion of mixed nests

Species	Population	*P* and TRT		Sampled nests	
		Reference	Number of incubations[a]	Year(s)[b] and reference	Number of nests
Caretta caretta	Brazil	Marcovaldi et al. 1997	6	1996 and 1997 (data from both years mixed together) (Mrosovsky et al. 1999)	51
	Florida	Mrosovsky 1988	4	1986 (Mrosovsky and Provancha 1989)	45
				1987 and 1988 (Mrosovsky and Provancha 1992)	76
	Georgia	Yntema and Mrosovsky 1982; Mrosovsky 1988	10		
	Greece	Mrosovsky et al. 2002	9		
	Heron Island	Limpus et al. 1985	10		
	Mon Repos	Limpus et al. 1985; Georges et al. 1994	16		
	North and South Carolina	Mrosovsky 1988	8	1979, 1980, and 1982 (Mrosovsky et al. 1984b)	18
	South Africa	Maxwell et al. 1988	10	1988 (Maxwell et al. 1988)	18
Chelonia mydas	Suriname	Mrosovsky et al. 1984a; Godfrey and Mrosovsky 2006	8	1980 (Mrosovsky 1982)	12
				1981 and 1982 (Mrosovsky et al. 1984a)	113
				1993 (Godfrey et al. 1996)	79
Chrysemys picta	Nebraska	Gutzke and Paukstis 1983; Etchberger et al. 1992; Packard et al. 1989	11		
	Tennessee	Bull et al. 1982b; Ewert et al. 1994	7		
	Wisconsin	Bull et al. 1982b; Bull and Vogt 1979	7		
Clemmys guttata		Ewert and Nelson 1991	4		
Dermochelys coriacea	Pacific coast, Costa Rica	Binckley et al. 1998	8	1994 (Binckley et al. 1998)	18
	French Guiana and Suriname	Rimblot et al. 1985; Rimblot-Baly et al. 1987	10	1981, 1983, 1984 and 1985 (Rimblot-Baly et al. 1987)	34
				1982 (Mrosovsky et al. 1984a)	29
				1993 (Godfrey et al. 1996)	27
Emys orbicularis	Brenne, France	Reviewed in Girondot 1999	16		
Eretmochelys imbricata	Antigua	Mrosovsky et al. 1992	9		
	Brazil	Godfrey et al. 1999	7		
Gopherus agassizi		Spotila et al. 1994; Lewis-Winokur and Winokur 1995	11		

Table 1 continued

Species	Population	*P* and TRT		Sampled nests	
		Reference	Number of incubations[a]	Year(s)[b] and reference	Number of nests
Gopherus polyphemus	Florida	Demuth 2001	7	Unknown (Bull 1985)	1
Graptemys caglei		Wibbels et al. 1991	5		
Graptemys ouachitensis	Wisconsin	Bull et al. 1982a, b; Bull and Vogt 1979	6	1982 (Demuth 2001)	58
Graptemys pseudogeographica	Tennessee	Bull et al. 1982b	7		
	Wisconsin	Bull et al. 1982b; Ewert and Nelson 1991	10	1982 (Demuth 2001)	9
Lepidochelys olivacea		Wibbels et al. 1998; McCoy et al. 1983	7		
Malacochersus tornieri		Ewert et al. 2004b	5		
Podocnemis expansa	Colombia	Valenzuela 2001	4	1993–1994 (Valenzuela et al. 1997)	6
				1997–1998 (Valenzuela 2001)	3
Podocnemis unifilis	Brazil	De Souza and Vogt 1994	6	1990 (De Souza and Vogt 1994)	15
Terrapene carolina		Ewert and Nelson 1991	8		
Testudo graeca		Pieau 1971, 1972, 1975	6		
Testudo hermanni		Eendebak 1995	8		
Trachemys scripta	Tennessee	Bull et al. 1982b; Ewert and Nelson 1991	7		

P Pivotal temperature, TRT transitional range of temperatures

[a] Number of different constant incubation temperatures used to derive the TSD parameters for the population

[b] The year indicates when the study took place in the field

Table 1). For a more precise description of mixed nest data, see Hulin et al. (2008).

Reaction norm parameters and the proportion of mixed nests

To test the relation between *P* and TRT values and the observed proportion of mixed nests, we used two complementary methods. First, the proportion of mixed nests was fitted using a logistic regression as a function of *P*, TRT, both with or without an interaction term. Maximum likelihood using a binomial link function was used. We mixed together all data available for each population, regardless of the year of the study because too few data exist to test for a year effect. Model selection was completed using AICc. The total number of individual nests used to estimate the mean proportion of mixed nests for a population varied from 1 nest for *Gopherus polyphemus* in Florida to 204 nests for *Chelonia mydas* in Suriname, but this factor was taken into account in the binomial link and did not influence the results. Each population was considered as independent for our statistical analysis. Indeed, because all species save two were represented by only one sampled population, it was impossible to take into account the 'population' level.

Second, a mechanistic model of TSD was used to test directly for the impact of TRT change on the proportion of mixed nests. The mechanistic model we used mimics the physiological mechanisms of temperature-dependent sex determination by taking into account changes in the growth rate of embryos as related to incubation temperature (Georges et al. 2005). Gonads exhibit the same growth as the embryo, modulated by the amount of estrogens in the gonads, which act as an inhibitor of gonad growth (Pieau et al. 1994). Estrogen is produced by the enzyme aromatase, and the amount of aromatase is also dependent on temperature, with higher levels present at higher temperatures. Finally, the direction of sex determination is defined by a threshold value of estrogens per unit of gonad (Vaillant et al. 2001). This

Table 2 Model scores according to the corrected Akaike information criterion (AICc) and values obtained for P and TRT, and associated standard errors (SE)

Species	Population	Model (AICc)[a]			P (°C) (SE)	TRT (°C) (SE)	Proportion of mixed nests	
		No_K	K_{fix}	K_{var}			Per year	Mean[c]
Caretta caretta	Brazil	22.13[b]	31.82	61.47	29,15 (0.07)	1.73 (0.29)	0.27 (1996)	0.27
	Florida	30.75[b]			29.11 (0.11)	3.24 (0.58)	0.13 (1986) 0.02 (1987) 0.5 (1988)	0.18
	Georgia	67.96	62.06[b]		28.69 (0.04)	3.06 (0.01)		
	Greece	19.76[b]	23.70	30.90	29.33 (0.08)	1.53 (0.30)		
	Heron Island	26.63	24.66[b]	30.52	29.33 (0.04)	4.77 (0.13)		
	Mon Repos	67.48	70.45	67.14[b]	28.64 (0.13)	5.49 (0.65)		
	North and South Carolina	36.58[b]	38.40	46.89	29.15 (0.01)	3.26 (0.01)	0 (1979) 0.50 (1980) 0.69 (1982)	0.61
	South Africa	12.04[b]	15.23	21.23	29.74 (0.08)	0.93 (0.28)	0.44 (1988)	0.44
Chelonia mydas	Suriname	41.20[b]	45.11	54.32	29.14 (0.18)	4.87 (0.99)	0.67 (1980) 0.71 (1981) 0.73 (1982) 0.63 (1993)	0.69
Chrysemys picta	Nebraska	73.99[b]	75.46	80.06	28.35 (0.11)	3.83 (0.42)		
	Tennessee	15.93[b]	21.99	35.85	27.40 (0.12)	1.21 (0.27)		
	Wisconsin	19.26[b]	20.63	34.62	29.05 (0.06)	1.28 (0.20)		
Clemmys guttata		28.37[b]			27.12 (0.49)	8.47 (1.95)		
Dermochelys coriacea	Pacific coast, Costa Rica	13.25[b]	18.85	27.98	29.43 (0.10)	1.18 (0.32)	0 (1994)	0
	French Guiana and Suriname	8.87[b]	13.16	19.16	29.51 (0.02)	0.08 (0.05)	0.5 (1986) 0.33 (1982) 0.37 (1994)	0.4
Emys orbicularis	Brenne, France	94.36	90.08	87.81[b]	28.51 (0.01)	1.00 (0.01)		
Eretmochelys imbricata	Antigua	18.91[b]	21.51	28.70	29.27 (0.05)	1.00 (0.16)		
	Brazil	31.12[b]	34.08	47.96	29.66 (0.07)	1.58 (0.26)		
Gopherus agassizi		10.41[b]	14.34	19.58	30.96 (0.06)	0.66 (0.21)		
Gopherus polyphemus	Florida	20.03[b]	26.34	39.62	29.34 (0.22)	4.22 (0.90)	1 (–[d])	1

Table 2 continued

Species	Population	Model (AICc)[a]			P (°C) (SE)	TRT (°C) (SE)	Proportion of mixed nests	
		No_K	K_{fix}	K_{var}			Per year	Mean[c]
Graptemys caglei		20.96[b]	38.11		29.65	2.02		
					(0.15)	(0.56)		
Graptemys ouachitensis	Wisconsin	20.79[b]	30.02	59.77	29.21	0.86	0.29 (1980)	0.29
					(0.02)	(0.14)		
Graptemys pseudogeographica	Tennessee	24.42[b]	30.32	43.32	29.01	1.56		
					(0.07)	(0.28)		
	Wisconsin	22.65	24.84[b]	30.46	29.41	1.38	0.33 (1980)	0.33
					(0.05)	(0.17)		
Lepidochelys olivacea		30.54	29.93[b]	43.77	31.04	2.96		
					(0.07)	(1.07)		
Malacochersus tornieri		12.12[b]	32.12		29.98	0.36		
					(0.10)	(1.05)		
Podocnemis expansa	Colombia	26.10[b]			32.14	4.86	0.83 (1993)	0.89
					(0.32)	(1.14)	1 (1997)	
Podocnemis unifilis	Brazil	34.29[b]	39.44		31.43	6.84	0.8 (1990)	0.8
					(0.30)	(1.39)		
Terrapene carolina		96.04	75.34[b]		27.84	7.56		
					(0.24)	(0.82)		
Testudo graeca		15.41[b]	22.38	52.20	30.36	0.95		
					(0.12)	(0.24)		
Testudo hermanni		13.49[b]	19.09	27.58	31.57	2.16		
					(0.15)	(0.53)		
Trachemys scripta	Tennessee	21.64[b]	28.35		29.16	2.19		
					(0.10)	(0.34)		

[a] Blank cells in AICc columns signify that the model could not fit the data

[b] Model selected according to AICc criterion

[c] Mean value of the proportion when nests from all years are mixed together

[d] Year of the field study unknown

model was parameterized using physiological data collected from eggs of the European pond turtle (*Emys orbicularis*) incubated at different constant temperatures and validated using a large range of constant and variable incubation temperatures (Delmas et al. 2008). For each simulated embryo, a set of parameters was obtained within a range of possible values based on empirical data. This simulated inter-individual variability accounted for potential factors such as maternal effects (Bowden et al. 2000), as well as genetic factors that may influence sexual differentiation in TSD reptiles (Pieau et al. 1999). Different values of TRT were produced by modifying the variability of the amount of estrogens in the eggs at the beginning of gonadal growth: the higher the variability, the larger the TRT values. TRT values used here were 0.43, 0.69, 0.75, 1.35 and 2.50°C. The model was run with nests containing 1, 3, 5, 10, 20, 30, 50 and 100 eggs. For each of these values, we repeated 100 simulations, to limit the error of sex ratio estimates, with 1,000 different fluctuating temperature regimes randomly defined from a daily mean temperature of 25.6 ± 2°C and an associated thermal variance of 5 ± 2°C. These theoretical ranges of temperature variations were chosen to approximately overlap the thermal incubation conditions encountered by nests of *Emys orbicularis*. Moreover, these conditions allowed sex ratio distributions to be centered approximately around 0.5. The results of the model simulations provided estimates of the theoretical distributions of the nest sex ratios under variable thermal conditions of incubation from which the proportion of unisexual nests was calculated. We then compared the simulated distributions under TSD with the simulated distributions obtained from a genotypic sex determination (GSD) mechanism.

Results

TSD reaction norm

For each population, a model was selected according to the AICc criterion and subsequently used to estimate values of TRT and *P* (Table 2). These values are variable both at inter- and intra-population levels. The number of data points was sometimes lower or equal to the number of parameters for K_{fix} and/or K_{var} models (*Caretta caretta* in Florida and Georgia, *Clemmys guttata*, *Graptemys caglei*, *Malacochersus tornieri* and *Podocnemis expansa* in Colombia), in which case we decided not to fit these two models. In some other cases (*Podocnemis unifilis*, *Terrapene carolina* and *Trachemys scripta* in Florida and in Tennessee), the fit of K_{var} models did not produce the expected TSD shape with the sex ratio ranging from 0 to 1. We therefore excluded these results.

Reaction norm parameters and the proportion of mixed nests under natural conditions

Based on the above, we selected data from 12 populations of 8 different species. The mean proportion of mixed nests obtained from our review was 0.44. However, this proportion varied greatly between populations: from 0 for *Dermochelys coriacea* in Costa Rica ($n = 18$) to 1 for *Gopherus polyphemus* in Florida ($n = 1$). In the two species for which we had data from different populations, the results varied from 0.18 (Florida, $n = 121$) to 0.61 (North and South Carolina, $n = 18$) for *Caretta caretta*, and from 0 (Costa Rica, $n = 18$) to 0.40 (French Guiana and Suriname, $n = 90$) for *Dermochelys coriacea*.

The proportion of mixed nests is dependent on *P*, TRT and their interaction (Table 3). *Podocnemis expansa* and *P. unifilis* showed high *P* values compared to other populations. We were suspicious that the distribution of points from the populations of *Podocnemis* may have been overly influential in the regression (Fig. 1). Therefore, we calculated the likelihood, AICc and Akaike weights of the models after excluding these two populations (Table 3). The selected model included both *P* and TRT but not the interaction. From this, we concluded that the correlation between the proportion of mixed nests and the interaction between *P* and TRT was driven primarily by the high values of *P* for the *Podocnemis* populations. Overall, we conservatively conclude that the proportion of mixed nests is positively correlated to TRT and, to a lesser extent, to *P* (Fig. 2).

Given that both *P* and TRT parameters have error values associated with them, we used a weighted ridge regression (Hoerl and Kennard 1970) to search for a relationship between *P* and TRT (Fig. 3). The limits of the confidence interval of the regression coefficient included the constant model (coefficient varying from −0.440 to 0.201). Therefore, we could not conclude that *P* and TRT were significantly correlated in our data.

Simulations from the mechanistic model showed that, for a given number of eggs in clutches, the proportion of mixed nests reaches a maximum value under a GSD mechanism (Fig. 4). The proportion of mixed nests increases with increasing values of TRT, and tends toward the proportion of mixed nests obtained under GSD. As expected from a sampling effect, the proportion of mixed nests is also positively correlated to the number of simulated eggs under TSD and GSD mechanisms (Fig. 4).

Table 3 Results of model fit and selection based on AICc and Akaike weight for the correlation between the proportion of mixed nests and *P*, TRT, and the interaction between *P* and TRT for all populations (a), and excluding data from *Podocnemis expansa* and *Podocnemis unifilis* (b)

(a) Parameters	Constant model	TRT only	*P* only	*P* and TRT	*P*, TRT and interaction
Constant	0.223	1.207	16.136	20.850	116.167
TRT parameter	0	−0.328	0	−0.334	−18.132
P parameter	0	0	−0.543	−0.670	−3.910
Interaction	0	0	0	0	0.603
AICc	179.622	127.833	172.0586	121.945	**108.306**
Akaike weight	$3.262.10^{-16}$	$5.743.10^{-5}$	$1.432.10^{-14}$	$1.091.10^{-3}$	**#1**
(b) Parameters	**Constant model**	**TRT only**	***P* only**	***P* and TRT**	***P*, TRT and interaction**
Constant	0.287	1.173	−22.659	111.119	141.534
TRT parameter	0	−0.307	0	−0.566	−40.930
P parameter	0	0	0.785	−3.736	−4.775
Interaction	0	0	0	0	1.384
AICc	158.616	119.142	159.338	**100.000**	103.746
Akaike weight	$1.620.10^{-13}$	$6.042.10^{-5}$	$1.129.10^{-13}$	**$8.668.10^{-1}$**	$1.332.10^{-1}$

Bold values represent the model selected according to the criteria for AICc or Akaike weight

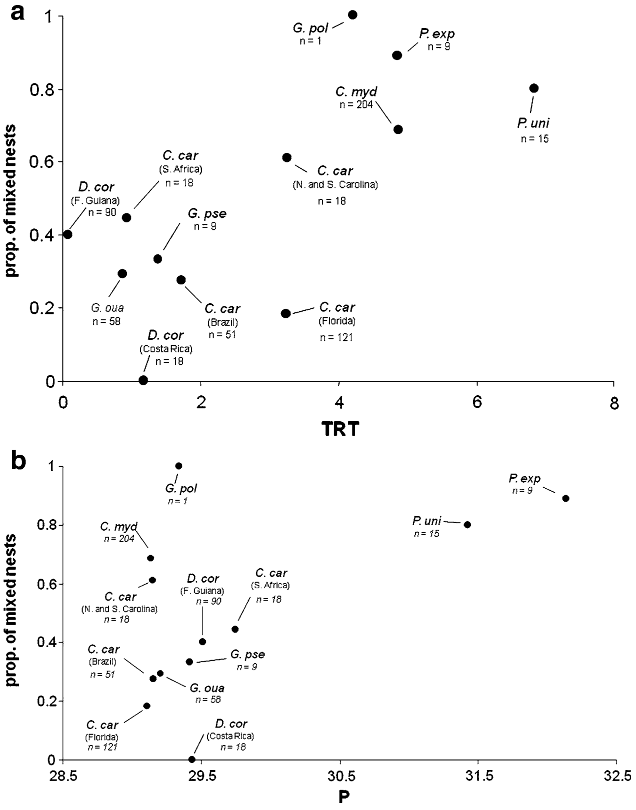

Fig. 1 Proportion of mixed nests in natural populations in relation to the value of **a** transitional range of temperatures (TRT; °C) and **b** pivotal temperature (*P*; °C). For each population considered, *n* is the total number of nests used to calculate the proportion of mixed nests

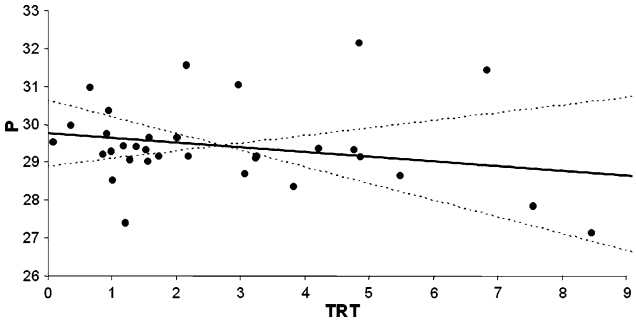

Fig. 3 Pivotal temperature (*P*) in relation to the value of TRT (°C). *Solid black line* Linear regression obtained with ridge correlation (TRT = −0.123 * *P* + 29.762), *dashed black lines* interval of confidence of the regression (slope from −0.440 to 0.201)

Discussion

Under predicted future climate scenarios, future environmental conditions are uncertain (Zwiers 2002) but it is widely accepted that warming will occur (IPCC 2007). Our study focuses on the potential impact of rising temperatures on turtle populations. As these species exhibit TSD, new thermal conditions may have a great impact on sex ratios and, in turn, on population dynamics and maintenance.

The modeled values of *P* and TRT we obtained were consistent with the corresponding values reported in the literature. For instance, in the case of sea turtles, we obtained values of *P* similar to those stated by Davenport (1997): all were close to 29°C, except for *Lepidochelys olivacea* (31.04°C). Note that the latter value is in accordance with Wibbels et al. (1998). Estimates for *Chrysemys picta*, *Emys orbicularis* and *Gopherus polyphemus* were also similar to previously reported values (Ewert et al. 1994; Girondot 1999). Comparable to Valenzuela (2001), we found a higher pivotal temperature for *Podocnemis expansa*. Although values of TRT are not always reported

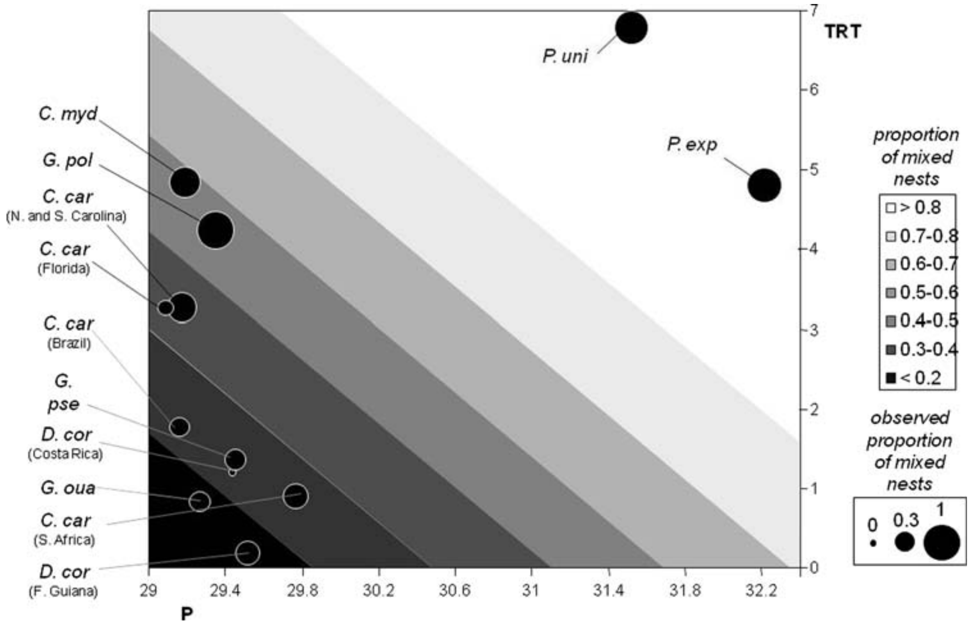

Fig. 2 Proportion of mixed nests predicted by the model selected by AICc in relation to *P* and TRT (Prop. of mixed nests = $\frac{1}{e^{20.85-0.334*TRT-0.67*P}}$). Each point represents a population. For each population, the surface of the *circle* is positively related to the observed proportion of mixed nests for each population

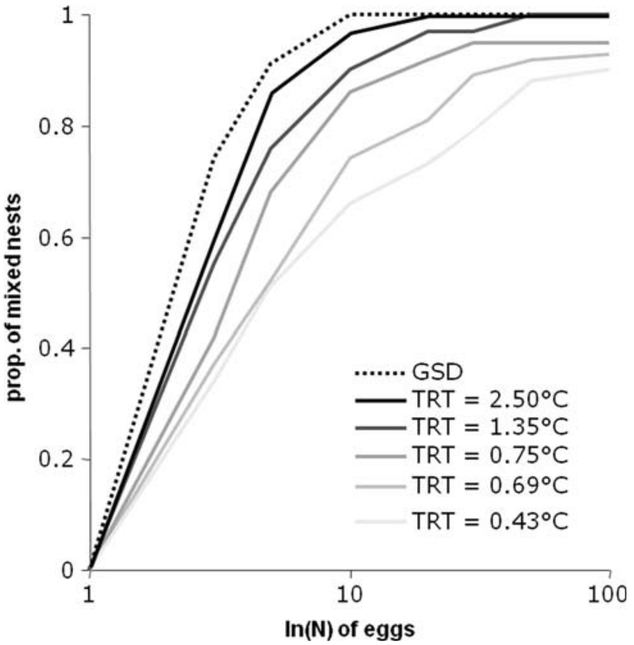

Fig. 4 Proportion of mixed nests in relation to the number of eggs in each clutch. The sex ratios are produced under a temperature-dependent sex determination (TSD) Ia mechanism with five different values of TRT, and under a genotypic sex determination (GSD) mechanism

in the literature, our results were consistent both with reported precise values (Girondot 1999) or indications (for instance, Valenzuela (2001) reported a sex ratio of 100% males at 29.5°C for *Podocnemis expansa*, which suggests a high pivotal temperature value). Our aim here was not to match exactly previously reported values. Indeed, there are general differences of *P* and TRT estimates across studies that are due largely to the different methods used for calculating these parameters (Ewert et al. 2004a). However, general similarities between our estimated values and published figures suggest that the models we used to calculate *P* and TRT values are satisfactory.

In the literature, the proportion of mixed nests is almost always based on a sub sample of hatching offspring (Hulin et al. 2008). As the probability of sampling offspring of both sexes from a mixed nest increases with the size of the sample (Fig. 4), our estimate of the proportion of mixed nests may be underestimated.

Our study showed a positive correlation between the proportion of mixed nests and the TRT value in the sample turtle populations. The correlation between TRT and the proportion of mixed nests is highly significant and the effect is biologically strong (from 0 to 100% of mixed nests). We also found an effect of *P* on the proportion of mixed nests. The positive correlation between the proportion of mixed nests and *P* could result from the fact that, in many turtle populations, unisexual nests are mostly female (Hulin et al. 2008). At equal TRT values, for TSD Ia species, a higher *P* is predicted to result in the conversion of female unisexual nests into mixed nests. Contrary to previous studies (Ewert et al. 2004a; Girondot 1999), we did not find that the value of *P* was significantly correlated to the value of TRT. This difference from previous studies is difficult to interpret. Indeed, the species studied by Girondot (1999) are included in the present study, but we used supplementary data from these or other species. The species studied by Ewert et al. (2004a) were largely not included in the present study, because it was impossible to correctly estimate values for *P* and TRT from the available data in the published literature. Increasing the datasets available for TSD parameters for various turtle species would facilitate future studies that may more accurately describe the relationship between *P* and TRT.

Simulations of the mechanistic model of TSD showed that the value of TRT may drastically influence the proportion of mixed nests under variable thermal conditions of incubation (Fig. 4). We showed that the probability of producing mixed nests increases when the value of TRT increases. We also showed that this probability increases when the number of eggs in clutches increases and reaches a maximum value. Finally, we found that, under a moderate range of variation in incubation temperatures, higher TRT values provided higher proportions of mixed nests that tend toward the proportion obtained under a GSD system (Fig. 4). Taken together, our results suggest strongly that higher proportions of mixed nests in populations may be a direct result of higher values of TRT.

Our conclusion may seem rather intuitive but it is not a logical deduction from the definition of TRT. The TRT value is based on data collected from the incubation of a mixed sample of eggs from various nests at constant incubation temperatures: it is defined at the level of a population and does not predict the sex ratio of individual nests. The TRT ignores the possibility that female turtles select the temperature of their nests in a non-random way with respect to temperature. Under laboratory conditions, TRT represents the range of constant incubation temperatures that yields both sexes in variable proportions. However, in natural conditions, the temperature of a nest often fluctuates during the entire period of incubation, including during the thermosensitive period, when sex determination occurs in TSD species. Temperature variations overlapping the TRT during the thermosensitive period are a necessary condition to produce both sexes in nests (Pieau 1982). Our results confirm this prediction as we here show that populations with a lower value of TRT should have a higher probability of producing unisexual nests under variable environments, compared to populations with higher TRT values.

Under climate change, a shift in thermal regimes of incubation may yield a bias of sex ratio in populations of

TSD species (Janzen 1994; Walther et al. 2002). To our knowledge, no previous study has considered the influence of TRT on natural sex ratios and on the evolution of TSD species in response to climate change. For populations with lower TRT values, the consequences to climate change could be dramatic if thermal conditions that allow differentiation of both sexes are no longer available for incubating eggs. However, TSD species could overcome climate change through several ways, such as experiencing a shift in the value of *P* and/or TRT through natural selection or the modification of nesting phenology and/or behavior (Doody et al. 2006).

In populations that experience greater biases in sex ratios due to climate change, genetic factors favoring the production of both sexes, and especially the rarer sex, would have a selective advantage. The ability of the TSD reaction norm to evolve accordingly would depend on the heritable genetic variation present in the population, and on the expression of this genetic variation (Bull et al. 1982a; Janzen 1992; Rhen and Lang 1998). The amount of variation, whether genetic or not, in any given population is presently unknown. This variation could be estimated by experimentally incubating clutches at the pivotal temperature and examining significant deviations of clutch sex ratios from 50:50, with the limitation that the estimated variation could result from either heritable genetic variation or maternal effects. Besides, a higher proportion of mixed nests would favor the expression of heritable genetic variation between embryos (Hulin et al. 2008), and thus indicate an increased evolutionary potential for the population. Regardless of the genetic or environmental factors affecting a particular population, we predict that populations with higher TRT should be more amenable in adapting to climate change.

Some studies have failed to show variations of *P* and TRT between populations despite differences in the local environments (Bull et al. 1982a; Ewert et al. 2005; Mrosovsky 1988; Vogt and Flores Villela 1992). Other authors have suggested that global change could be too rapid for an evolution of *P* to occur (Freedberg and Wade 2001; Morjan 2003a; Nelson et al. 2004). As an alternative to *P* and TRT evolution, impacts of climate change on TSD species could be mediated by local adaptation through maternal effects, such as oestrogen concentrations in eggs (Bowden et al. 2004) or nest-site choice (Ewert et al. 2004a; Morjan 2003b). Nest-site selection could also evolve as a genetically determined trait, although in at least one turtle species this behaviour apparently evolves more slowly than the reaction norm to temperature (Morjan 2003b). Also, it remains to be seen if individuals could alter the timing of reproduction, to take advantage of different incubation conditions outside the normal nesting season. A few studies have addressed this question in sea turtle populations (Hawkes et al. 2007; Weishampel et al. 2003), but more analyses and long-term datasets are needed. Despite all these uncertainties about the evolution of maternal effects, it could be another avenue for adaptation for TSD populations with low TRT values.

To predict the consequences of modified thermal conditions on the sex ratios of TSD species, it is necessary to explore the link between temperatures and the sex ratio under natural conditions. Because direct and accurate observation of the sex ratio of TSD turtle offspring requires histology and is thus logistically difficult to carry out (Mrosovsky and Benabib 1990; Whitmore et al. 1985), many authors have developed models of prediction of sex ratios under natural conditions. Our results suggest that taking only *P* and not TRT into account may introduce errors into the estimates of nest sex ratios generated by these indirect methods. Indeed, the proportion of mixed nests in the population is positively linked to the value of TRT, or to the value of both TRT and *P*. By focusing only on *P*, as some methods do, the implicit assumption is that the TRT has a value of zero. In these cases, the proportion of mixed nests would likely be underestimated. The consequences of not including information on the TRT when estimating population sex ratios are unclear, but our results suggest greater caution is needed, especially when studying past or future sex ratios (Glen and Mrosovsky 2004; Hawkes et al. 2007). In any case, taking TRT into account in these models does not remove the issue of comparing laboratory derived values with natural conditions. An alternative approach is to use a mechanistic approach, such as that taken in this study, and which has provided valuable results in studies on *Emys orbicularis* (Delmas et al. 2008).

Overall, we stress the importance of taking TRT into account when considering the dynamics of a population under natural conditions. It is essential to include values of TRT in correlative models of sex ratios under natural conditions. Moreover, when considering the impact of global change on TSD species, TRT may be linked to the risk of extinction. Further work in this area is needed. In particular, studies should focus on assessing the ability of TSD species to adapt to changing environments. This would greatly increase our ability to predict how TSD species may survive in response to different possible scenarios of global warming.

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