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Evolution and Maintenance of Temperature-Dependent Sex Determination

Temperature-dependent sex determination (TSD) is an ancient sex-determining mechanism among vertebrates, perhaps ancestral in reptiles. How and why transitions between TSD and genotypic sex determination (GSD) occur are two important questions regarding TSD origin and maintenance. Most formulated hypotheses for TSD evolution are adaptive, but neutral or quasi-neutral alternatives exist as well (Table 14.1). Here possible explanations for the origin and persistence of TSD are reviewed, and these evolutionary events are treated separately because each may result from different forces. First, the postulated adaptive and neutral hypotheses are described, the existing evidence (or lack thereof) for TSD taxa is explored, and a series of testable predictions based on theory is presented. The third section briefly discusses TSD absence in various vertebrate groups. In general, current hypotheses deal with genetic, maternal, and environmental effects on fitness components, either survival or fertility, sometimes overlapping several effect types (Figure 14.1).

Theory and Evidence for TSD Taxa

This section considers transitions from GSD to TSD (TSD gains) and from TSD to GSD (TSD loses), TSD persistence and differentiation, and whether each of these events can be explained by chance or selection.

TSD Origin: From GSD to TSD

Adaptive Hypotheses

Theoretically, selection favors equal parental investment in male and female production, and if the costs of producing one male and one female are identical, the sex ratio tends to equality (Fisher 1930). But when these costs differ, a biased sex ratio can equalize total parental investment in male and female production, yield a higher reproductive success than the average under balanced sex ratios, and thus be selected (Shaw and Mohler 1953). Consequently, a mechanism allowing such biases is favored.

In TSD, males and females are produced polyphenically—identical genomes can produce discrete phenotypes depending on the environmental conditions (e.g., incubation temperature). Polyphenisms are a form of phenotypic plasticity that may help coping with heterogeneous environments (Kawecki and Stearns 1993 in Downes and Shine 1999). At first glance the life history and ecology of some TSD and GSD taxa appear very similar, yet their sex-determining mechanisms differ. Seasonal sex ratio shifts may be selected when life histories vary seasonally (but differently) for males and females, and if generations overlap causing individuals from different cohorts to compete for reproductive success (Werren and Charnov 1978). Under those circumstances, parental ability to bias sex ratios accordingly is also favored.

Table 14.1 Evidence Related to the Adaptive and Neutral Hypotheses Described in the Text

Theoretical Models	Theoretical Models	Evidence in Pro	Evidence Against	Equivocal Evidence
TSD Origin				
Adaptive				
Differential fitness	Charnov and Bull 1977			
Maternal Effects				
Nest-site choice				
Natal homing	Reinhold 1998	Reinhold 1998	Valenzuela and Janzen 2001	
Differential dispersal	Julliard 2000		Godfrey et al. 1996 Mrosovsky 1994 Valenzuela and Janzen 2001	
Survival				
Differential mortality	Burger and Zappalorti 1988	Janzen 1995	Rhen and Lang 1995	Bobyn and Brooks 1994 Elphick and Shine 1999 Gutzke and Packard 1987a Joanen et al. 1987
Fecundity				
Sexual size dimorphism	Head et al. 1987 Webb et al. 1987	Allsteadt and Lang 1995a Crews et al. 1998 Ferguson and Joanen 1982 Packard and Packard 2001 Rhen and Lang 1995 Saillant et al. 2002 Tousignant and Crews 1995	Allsteadt and Lang 1995a O'Steen and Janzen 1999 St. Clair 1998 Steyernark and Spotila 2001	Bobyn and Brooks 1994 Braña and Ji 2000 Campos 1993 Demuth 2001 Elphick and Shine 1999 Ewert et al. 1994 Ferguson and Joanen 1983 Gutzke and Packard 1987a Gutzke et al. 1987 Harlow and Shine 1999 Hutton 1987 Joanen et al. 1987 McKnight and Gutzke 1993 O'Steen 1998 Packard et al. 1987 Webb and Cooper-Preston 1989
Seasonal hatching time	Conover and Kynard 1981	Conover and Kynard 1981 Harlow and Taylor 2000		

Phenotypic effects		Allsteadt and Lang 1995a Crews et al. 1998 Gutzke and Crews 1988 Rhen and Lang 1999b	Arnold et al. 1995 Bull and Charnov 1989 Packard and Packard 2001	Braña and Ji 2000 Demuth 2001 Elphick and Shine 1999 Gutzke et al. 1987 Janzen 1995 O'Steen 1998 O'Steen and Janzen 1999 Packard et al. 1987 Tousignant and Crews 1995 Webb et al. 2001
Biased sex ratios				
Group structure adaptation	Bull and Charnov 1988		Burke 1993 Thorbjarnanson 1997	
Neutral				
Preexisting T°sensitivity	Bull 1981			Chardard et al., Chapter 7
Sex-ratio distorter	Morjan 2002	–	–	–
TSD Maintenance				
Adaptive				
Maternal Effects				
Nest-site choice by egg size	Roosenburg 1996	Bull 1983 Roosenburg 1996	Bulmer and Bull 1982 Ewert and Nelson 1991 Valenzuela 2001a,b	
Biased sex ratios				
Group selection of sex ratio	Woodward and Murray 1993		Girondot and Pieau 1996 Thorbjarnanson 1997	Woodward and Murray 1993
Sib-avoidance	Ewert and Nelson 1991		Burke 1993	
Cultural inheritance of natal homing	Freedberg and Wade 2001		Godfrey et al. 1996 Valenzuela and Janzen 2001	Gibbons 1990 Mrosovsky 1994
			Bull 1980 Bull and Charnov 1988	Thorbjarnarson 1997
Neutral				
TSD equivalent to GSD	Bull 1980 Mrosovsky 1980			
Phylogenetic inertia	Bull 1980	Ewert and Nelson 1991	Burke 1993 Janzen and Paustkis 1991b	
Life-history dependent				
Longevity	Bull and Bulmer 1989 Girondot and Pieau 1996	Bull and Bulmer 1989 Girondot and Pieau 1996 Girondot and Pieau 1999		

(*continues*)

Table 14.1 (*Continued*)

Theoretical Models	Theoretical Models	Evidence in Pro	Evidence Against	Equivocal Evidence
Overlapping generations	Bull and Bulmer 1989	Bull and Bulmer 1989		
	Girondot and Pieau 1996	Girondot and Pieau 1996		
		Girondot and Pieau 1999		
TSD Loss				
Adaptive				
GSD individuals more fit	Bull 1981	de Lisle 1996	Robert and Thomson 2001	
	Bull 1983			
Sex ratio fluctuations	Bull 1980	Conover and Heins 1987a	Post et al. 1999	
		Godfrey et al. 1996		
Intersexes	Bull 1981	Pieau 1982	Bull 1983	
		Pieau et al. 1999a,b	Crews et al. 1998	
			Girondot et al. 1998	
Allee effect	Berec et al. 2001			Berec et al. 2001
Low/late dimorphism	Bull 1983	Greenbaum and Carr 2001		
		Gutiérrez et al. 2000		
Antagonistic pleiotropy	Moran 1992			
Imperfect phenotype-environment matching	Moran 1992			
Parental sex ratio control	Roosenburg 1996		Bull 1980	
	Reinhold 1998		Julliard 2000	
Neutral	–	–	–	–

Note : Theoretical models list the original source(s) of each particular hypothesis. *Evidence in Pro* refers to empirical data supporting any given hypothesis. *Evidence Against* refers to empirical data or theoretical considerations against each hypothesis. *Equivocal Evidence* refers to empirical data that can be interpreted either way because (a) no directional effect was detected, (b) data are in the expected direction but come from GSD taxa, or (c) experimental design or statistical difficulties preclude a clear conclusion. The evidence included in this table is not exhaustive.

GSD species can bias sex ratios adaptively (Bulmer and Bull 1982; Bull and Charnov 1988; Krakow 1992; Perret 1996; Komdeur et al. 1997; Nager et al. 1999), but sometimes at a relatively high cost (e.g., waste of resources invested in the wrong-sex offspring by differential offspring mortality or discarding of gametes). Higher benefits are needed to offset these costs; otherwise, sex ratio biases in GSD species would be constrained. When benefits are not large enough, a low-cost system allowing sex biases will be advantageous. This may be the case of TSD for some taxa. A key corollary of this scenario, however, is that when the benefits of biasing sex ratios are small, formidable efforts may be required to detect them.

1. DIFFERENTIAL FITNESS HYPOTHESIS

Charnov and Bull (1977) proposed that environmental sex determination (ESD) is selected over GSD if three conditions are met (the Charnov-Bull model): (1) the environment consists of patches (spatial or temporal) that grant sex-specific fitness to the offspring, (2) patches cannot be chosen by the offspring nor by their parents, and (3) random mating occurs among patches. When applied to TSD, thermal conditions early in development (when sex is determined) are assumed to differ unpredictably among patches and to confer differential lifetime fitness directly or through a correlated variable. This makes TSD beneficial, since offspring develop into the sex with highest fitness in each patch. Primary population sex ratios can deviate from ½ towards the lower-fitness sex through frequency-dependent selection plus the fitness differential of the patches (Bull 1983).

Environmental effects on development and fitness can be important to maintaining polyphenisms (Moran 1992). First, an environmental factor (cue) affects development, resulting in plasticity (sex-by-temperature production in TSD), while a factor (selective agent) affects fitness differently for alternative phenotypes. The cue can differ from the selective agent but must act first. The cue accuracy in predicting the selective-agent state (along with spatial vs. temporal environmental variation, fitness differentials among environments, relative frequency of the environments, and the cost of plasticity) determines the adaptive maintenance of phenotypic plasticity (Moran 1992). Consistently, under the Charnov-Bull model, sex ratio adjustment is selected first, and consequently, a mechanism allowing such adjustment is favored (Bull 1983).

The Charnov-Bull model is the most theoretically robust of the adaptive hypotheses, and has empirical support for some ESD invertebrates(e.g., Blackmore and Charnov 1989) and some vertebrate species (Table 14.2), although reptilian evidence is not conclusive, as will be shown below. Several related (sub)hypotheses (see TSD Origin, Differential Fitness Subhypotheses A–F, below) link incubation temperature with a fitness correlate to explain TSD origin or maintenance (see TSD Maintenance, Adaptive Hypotheses, Maternal effects on egg allocation).

Differential Fitness Subhypotheses Related to Maternal Effects by Female Nest-Site Choice

Natal homing. Natal homing could trigger ESD evolution if daughters are produced in high quality (i.e., high survival) sites to which they return to nest, thus deriving higher fitness than sons whose reproductive output is unaffected by natal patch quality (assuming they survive to maturity) (Reinhold 1998). Long-term fluctuations in nest-site quality and imperfect natal homing prevent runaway selection of nesting in high quality sites (Reinhold 1998). *Eretmochelys imbricata* exhibit natal homing, and offspring survival is positively correlated with percent females within nests (Swingland et al. 1990; Horrocks and Scott 1991), thus supporting this hypothesis (Reinhold 1998). This model was not applicable to *Chrysemys picta*, as hatching success and sex ratio were not repeatable, females did not nest preferentially in female-producing sites, and mortality was lower at male-producing sites (Valenzuela and Janzen 2001). This model is inapplicable to species whose lifespan is shorter than the scale of fluctuations in nest-site quality, otherwise runaway selection restricts nesting to high quality sites, leading to high female biases and thus to selection for GSD (Bull 1980).

Differential dispersal. Differential dispersal of males and females, and varying environmental quality (reproductive success) were proposed to explain the evolution of habitat-dependent biased sex ratios (Julliard 2000). The evolutionary stable strategy (ESS) overproduces the dispersing sex in poor habitats and the philopatric sex in good habitats, and biases increase in (1) rare or poor habitats, (2) under large habitat quality differentials, (3) under almost random dispersal of one sex regarding habitat availability, (4) with high (but different) dispersal rates for both sexes, (5) without individual control of reproductive habitat (Julliard 2000). The model purportedly supports the Charnov-Bull model by advocating differential fitness by environmental heterogeneity, and requires temporal predictability of habitat quality. However, no data exist on repeatability of nesting conditions in sea turtles (Mrosovsky 1994; contra Julliard 2000), and annual sex ratio can vary substantially in several turtle species (e.g., Godfrey et al. 1996; Valenzuela and Janzen 2001).

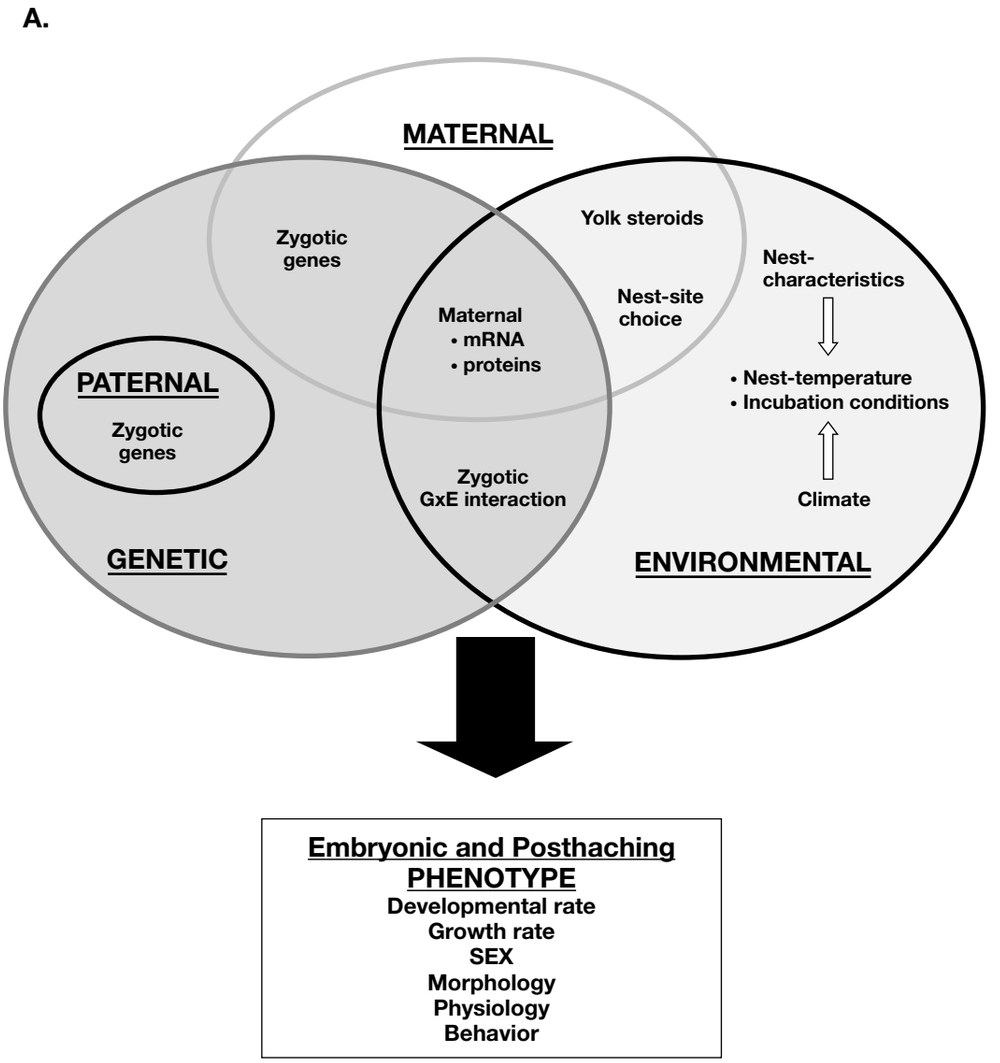
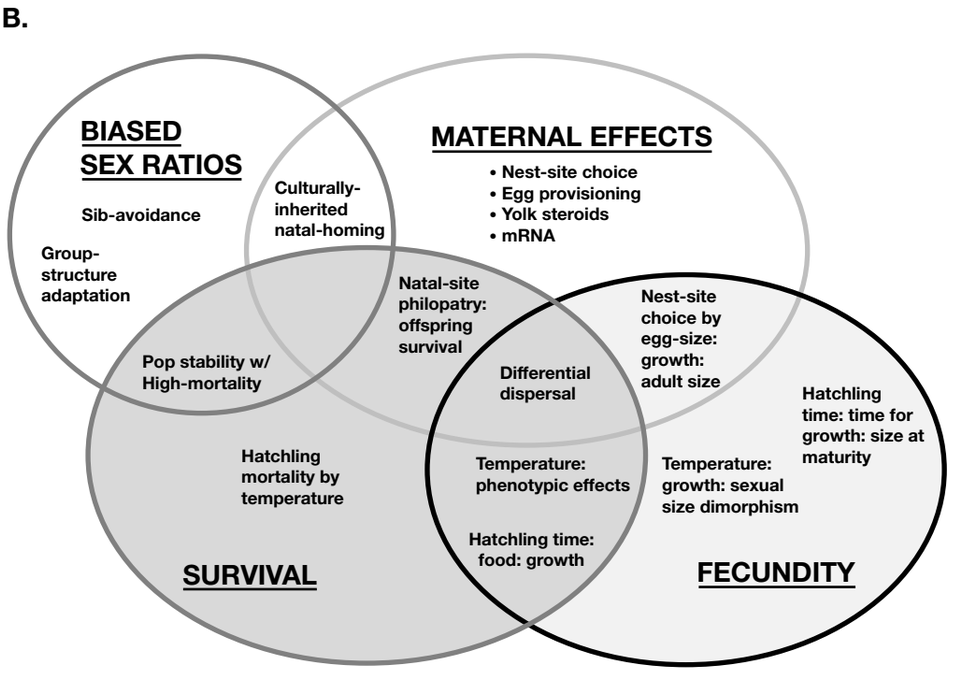


Figure 14.1 Diagram of the categories of factors affecting hatchling phenotype and classification diagram of TSD evolutionary hypotheses by the main fitness effect they address. (**A**) Factors with phenotypic effects on TSD individuals include genetic and environmental variables. Maternal effects encompass both types, whereas known paternal effects are only genetic. (**B**) Effects on lifetime reproductive success can be exerted through effects in survival (individuals must survive to maturity in order to reproduce) and/or fecundity (direct offspring production). Most postulated hypotheses for TSD evolution can be categorized by the fitness component they address, while a few other hypotheses focus primarily on biased sex ratios.

Nonetheless, this hypothesis requires direct testing at the relevant spatial scale.

Differential Fitness Subhypothesis Related to Survivorship

Differential mortality. Sex and incubation temperature in TSD could interact through a mechanism to enhance embryo survivorship (Webb and Smith 1984). If males and females suffer differential mortality during development that covaries with incubation temperature, TSD would be beneficial by allowing the production of the best-fit sex at the temperature extremes (Burger and Zappalorti 1988). Differential mortality may favor TSD origin, but once established, producing a single sex at extreme temperatures erases the traces of this pressure. The same applies to any differential fitness effect at single-sex temperatures. Therefore, evidence supporting this hypothesis requires artificially producing rare-sex individuals at the temperature ex-



tremes (e.g., Rhen and Lang 1995), which are predicted to suffer higher mortality than common-sex individuals at a given temperature. Interestingly, support for this hypothesis is equivocal as it comes from GSD species (Tables 14.1 and 14.2). No sex or temperature effect on hatchling mortality was found in snapping turtles using hormonal manipulations (Rhen and Lang 1995; contra Janzen 1995; but see Elphick and Shine 1999). Thus, this hypothesis does not explain TSD maintenance generally. Furthermore, data from intermediate temperatures (e.g., Janzen 1995) need not reflect relative fitness at single-sex temperatures (nor reveal whether producing one sex at such values is adaptive), because for each sex fitness can be higher, equal, or lower at mixed-sex-ratio temperatures than at extreme temperatures while sex ratio remains identical (Figure 14.2).

Differential Fitness Subhypotheses Related to Fecundity

Sexual size dimorphism. The sexual size dimorphism hypothesis considers differential temperature effects on growth, with the larger sex being produced at the optimal temperature (Head et al. 1987; Web et al. 1987; Deeming and Ferguson 1988; Ewert and Nelson 1991). Some data are consistent with this hypothesis (Ewert and Nelson 1991; Ewert et al. 1994). However, this evidence is not conclusive, partly because data amenable to analysis are reduced due to missing information from many taxa, and to confounding phylogenetic effects (Janzen and Paukstis 1991b; Mrosovsky 1994). As more species are studied and stronger phylogenies constructed, a reassessment of this hypothesis will prove valuable.

Seasonal hatching time. In some taxa temperature during development predicts seasonal hatching time and is associated with available time for growth before breeding and adult body size, which affects reproductive success differently for males and females (Conover and Kynard 1981; Conover 1984). In this case, TSD allows matching offspring sex with the hatching time during a breeding season that best affects fitness (Conover and Kynard 1981). This occurs in the fish *Menidia menidia* and *M. peninsulae* (Conover and Kynard 1981; Middaugh and Hemmer 1987; Conover, Chapter 2). GSD replaces TSD in northern populations of *M. menidia* where annual sex ratio fluctuations induced by climate cancel any fitness gain attained by TSD (Conover and Heins 1987b). Similarly, GSD prevails in subtropical semiannual populations of *M. peninsulae* (where temperature is a poor predictor of time for growth and size at maturity), and TSD increases with latitude as life cycle becomes annual (Yamahira and Conover 2003).

Comparable systems may be present in reptiles (Shine 1999; Harlow and Taylor 2000) as predictable seasonal temperature and sex ratio changes exist in some species (Mro-

Table 14.2 Examples of Existing Evidence Related to the Potential Adaptive Temperature Differential Fitness Effects

Temperature Effects	Species	Evid	Effect	Reference
Mortality	*Crocodylus porosus*	Eqv	T° or sex? MT	Webb and Cooper-Preston 1989
	Alligator mississippiensis	Eqv	T° or sex? ST	Joanen et al. 1987
	Chelydra serpentina	Eqv	T° or sex? MT	Bobyn and Brooks 1994
	Chelydra serpentina	Con	No T°, no sex, C, T°×Sex	Rhen and Lang 1995
	Chelydra serpentina	Pro	T°, sex, T°×Sex, ST	Janzen 1995
	Emydoidea blandingii	Eqv	T° or sex?	Gutzke and Packard 1987a
	Bassiana duperreyi^{GSD}	Eqv	T°, sex, T°×Sex, MT	Elphick and Shine 1999
Morphology	*Crocodylus porosus*	Eqv	T°, sex n.e., ST	Webb and Cooper-Preston 1989
(shape or size)	*Alligator mississippiensis*	Eqv	T°, sex, T°×Sex, ST	Ferguson and Joanen 1982
	Alligator mississippiensis	Eqv	T° or sex? MT	Ferguson and Joanen 1983
	Alligator mississippiensis	Eqv	T°, sex, T°×Sex, ST	Joanen et al. 1987
	Chlamydosaurus kingii	Eqv	T°, no sex, ST	Harlow and Shine 1999
	Chelydra serpentina	Con	C×T°, no T°, C, C×H, ST	Rhen and Lang 1999b
	Chelydra serpentina	Eqv	T° or sex? C, ST	McKnight and Gutzke 1993
	Chelydra serpentina	Eqv	T°, no sex, no T°×Sex, C, ST	O'Steen 1998
	Emydoidea blandingii	Eqv	T° or sex?	Gutzke and Packard 1987a
	Chelydra serpentina	Con	T°, no sex, no T°×Sex, ST	O'Steen and Janzen 1999
	Chelydra serpentina	Eqv	T° or sex?	Packard et al. 1987
	Alligator mississipiensis	Pro	T°, sex, T°×Sex, C	Allsteadt and Lang 1995a
	Podarcis muralis^{GSD}	Eqv	T°, sex	Braña and Ji 2000
	Terrapene carolina	Con	Growth unexpected by TSD mode	St. Clair 1998
	Terrapene ornata	Con	Growth unexpected by TSD mode	St. Clair 1998
	Chrysemys picta	Pro	No T°, Sex n.e., C, N	Packard and Packard 2001
	Chrysemys picta	Eqv	T° or sex?	Gutzke et al. 1987
	Eublepharis macularius	Pro	T°, sex, LT	Tousignant and Crews 1995
	Eublepharis macularius	Pro	T°, sex, LT	Crews et al 1998
	Bassiana duperreyi^{GSD}	Eqv	T°, sex, T°×Sex, MT	Elphick and Shine 1999
	Crocodylus niloticus	Eqv	T°, T°×C, no sex, MT	Hutton 1987
	Caiman crocodilus yacare	Eqv	T°, sex? n.e. directly	Campos 1993
	Gopherus polyphemus	Eqv	T°, no sex, ST	Demuth 2001
Growth	*Crocodylus porosus*	Eqv	T°, sex, T°×Sex, MT	Webb and Cooper-Preston 1989
	Alligator mississippiensis	Eqv	T°, sex, T°×Sex, ST	Ferguson and Joanen 1982
	Alligator mississippiensis	Eqv	T° or sex? MT	Ferguson and Joanen 1983
	Alligator mississippiensis	Eqv	T°, sex, T°×Sex, MT	Joanen et al. 1987
	Emydoidea blandingii	Eqv	T° or sex? ST	Gutzke and Packard 1987a
	Terrapene carolina	Con	Growth unexpected by TSD mode	St. Clair 1998
	Terrapene ornata	Con	Growth unexpected by TSD mode	St. Clair 1998
	Chelydra serpentina	Con	No T° or sex, C, ST	Steyermark and Spotila 2001
	Chelydra serpentina	Eqv	T° or sex? MT	Bobyn and Brooks 1994
	Chelydra serpentina	Pro	T°, no sex, C, T°×C, ST	Rhen and Lang 1995
	Chelydra serpentina	Eqv	T° or sex? C, Soc, ST	McKnight and Gutzke 1993
	Chelydra serpentina	Eqv	T°, no sex, no T°×Sex, ST	O'Steen 1998
	Eublepharis macularius	Pro	T°, sex, C, LT	Crews et al. 1998
	Dicentrarchus labrax	Pro	T°, Sire, Dam, S×T°,D×T°	Saillant et al. 2002
	Bassiana duperreyi^{GSD}	Eqv	T°, sex, T°×Sex, MT	Elphick and Shine 1999
	Gopherus polyphemus	Eqv	T°, no sex. ST	Demuth 2001
Energy reserves				
Res. yolk mass	*Chelydra serpentina*	Pro	T°, no sex, C, C×T°, ST	Rhen and Lang 1999b
	Chrysemys picta	Eqv	T° or sex?	Gutzke et al. 1987
Fat body/yolk mass	*Chelydra serpentina*	Eqv	T° or sex?	Packard et al. 1987
	Chrysemys picta	Con	No T°, sex n.e., C, N	Packard and Packard 2001
	Alligator mississipiensis	Pro	T°, sex, C	Allsteadt and Lang 1995a

Temperature Effects	Species	Evid	Effect	Reference
	Chelydra serpentina	Pro	T°, sex, C, ST	Rhen and Lang 1999b
	Chrysemys picta	Con	No T°, sex n.e., C, N	Packard and Packard 2001
	Alligator mississipiensis	Con	C×Sex, T°×C	Allsteadt and Lang 1995a
Behavior and performance	*Eublepharis macularius*	Pro	T°, sex, LT	Gutzke and Crews 1988
	Tropidonophis mairii^{GSD}	Pro	T°, sex, ST	Webb et al. 2001
	Eublepharis macularius	Pro	T°, sex, LT	Crews et al. 1998
	Chelydra serpentina	Eqv	T°, sex, T°×Sex, ST	Janzen 1995
	Chelydra serpentina	Eqv	T° , no sex, no T°×Sex, ST	O'Steen 1998
	Thamnophis elegans^{GSD}	Con	No T°, no sex, litter, no dam	Arnold et al. 1995
	Podarcis muralis^{GSD}	Eqv	T°, no sex	Braña and Ji 2000
	Bassiana duperreyi^{GSD}	Eqv	T°, sex, T°×Sex, MT	Elphick and Shine 1999
	Gopherus polyphemus	Eqv	No T°, sex, ST	Demuth 2001
Physiology	*Eublepharis macularius*	Pro	T°, sex, LT	Gutzke and Crews 1988
	Chelydra serpentina	Pro	T°, sex, C, ST	Rhen and Lang 1999b
	Chelydra serpentina	Eqv	T°, no sex, no T°×Sex, ST	O'Steen and Janzen 1999
	Eublepharis macularius	Pro	T°, sex, LT	Crews et al 1998
Reproductive success	*Eublepharis macularius*	Eqv	♀: no T°	Tousignant and Crews 1995
	Eublepharis macularius	Pro	T°, sex, LT	Gutzke and Crews 1988
Not reported	*Trachemys scripta*	Con	No effect	Bull and Charnov 1989

Note: Evid = evidence for the differential fitness hypothesis: Pro = evidence in support, Con = evidence against, Eqv = equivocal evidence (due to e.g., confounding factors, ultimate effect on fitness not known, or if species has GSD). Significant effects detected: T° = temperature, T° or sex? = temperature and sex effects confounded, T°×Sex = temperature by sex interaction, N = nest environment, C = clutch identity, T°×C = temperature by clutch interaction, C×Sex = clutch by sex interaction, Dam = maternal identity, Sire = paternal identity, S×T° = sire by temperature interaction, D×T° = dam by temperature interaction, Soc = social environment. No T°, no sex, or no T°×Sex = factors analyzed and found to have no significant effect. n.e = factor not explored in study. ST = short-term study (< 1 year for long-lived species), MT = medium-term study (at least 1.5–2 years for long lived species), LT = long-term study (> 2 years or up to maturity of short-lived species). The evidence presented in this table is not an exhaustive list.

sovsky et al. 1984b; Vogt and Bull 1984; Mrosovsky 1994; Godfrey et al. 1996; Bowden et al. 2000; Harlow and Taylor 2000; Girondot et al. 2002). For instance, increasing temperature during the nesting season correlates with increasing female offspring in the fast-growing, short-lived jacky dragon (Harlow and Taylor 2000). Early hatchlings are male, and grow for a longer time, thus attaining larger sizes than females. This is probably favored by sexual selection (Harlow and Taylor 2000). The possibility of temporal rather than spatial patches (sensu Charnov and Bull 1977) remains unexamined for long-lived species, perhaps because among-year temperature variation is generally viewed as causing undesirable sex ratio fluctuations, though longevity and overlapping generations buffer sex ratio variation among cohorts.

Phenotypic thermal effects. The remaining hypothesis predicts phenotypic thermal effects (e.g., morphology, physiology, or behavior; Table 14.2) independently of offspring sex or differentially in sons and daughters (e.g., Rhen and Lang 1995; Shine et al. 1995; Tousignant and Crews 1995; Shine 1999). Exploring these alternatives requires decoupling temperature from sex effects (Rhen and Lang 1995, Chapter 10), preventing individual thermoregulation and social interactions that can obscure thermal effects (Steyermark and Spotila 2001). Unfortunately, such decoupling is rarely done and long-term thermal effects on lifetime reproductive success are understudied (Table 14.2). Alternatively, phenotypes within sex across temperatures are compared assuming that fitness for each sex should be maximal at temperatures that only produce that sex and lower at values that produce both sexes, which may be incorrect (see Adaptive Hypotheses, Differential Mortality, above, and Figure 14.2). Support for this hypothesis is consistent but not necessarily conclusive (Table 14.2; Rhen and Lang, Chapter 10), particularly if no null hypothesis is falsified. Support from GSD species (Table 14.2) is also equivocal since they lack TSD despite displaying conditions purportedly favoring its evolution. Although heteromorphic sex chromosomes and TSD may not coexist in the same individual (Bull 1983; Valenzuela et al. 2003), evolving TSD from heteromorphic sex chromosomes is not precluded (Bull 1981; see TSD Origin, Adaptive Hypotheses; contra Elphick and Shine 1999). Further, contrary to Shine et al. (1995), theoretical models do not predict differential phenotypic effects of temperature only

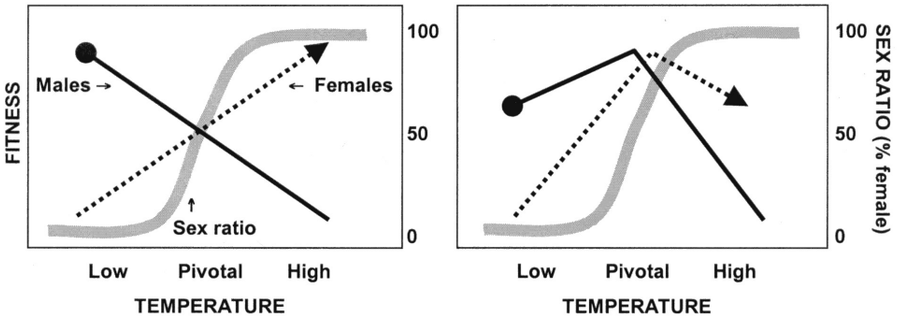


Figure 14.2 Hypothetical relationships between fitness and temperature for both sexes (black solid and dashed lines), and sex ratios (gray curve) that may be observed among or within species. As illustrated, identical sex ratios patterns could be expected from very different fitness within each sex. Solid circles and triangles denote the fitness value of the sexes that are produced at the extreme temperatures, while the opposite ends indicate the fitness of the sex that is no longer produced at the extreme temperatures. The pattern depicted here can be interpreted as TSD Ia (current illustration) or Ib (if solid line were to represent females and dashed line males, and the sex ratio axis were percent males). These simple examples could be extended to many other, more complex patterns.

in TSD species. Rather, theory proposes that differential thermal effects (sensu Charnov and Bull 1977) were either present in a GSD ancestor of TSD and subsequently favored TSD evolution, or are currently present and explain TSD persistence adaptively. Therefore, differential effects in GSD species constitute an opportunity to identify (1) potential effects for their TSD relatives, and (2) constraints preventing TSD evolution.

2. Adaptive hypotheses related to selection for biased sex ratios

Alternatives to the Charnov-Bull model's differential fitness hypothesis have also been proposed, some of which are also adaptive.

Group-structured adaptation. The group-structured adaptation hypothesis states that if populations are geographically structured into small breeding kin groups with minimal gene flow and refounded periodically, TSD allows the production of female-biased sex ratios, which would favor families or larger groups (sensu Hamilton 1967; Ferguson and Joanen 1983; Bull and Charnov 1988; Ewert and Nelson 1991). This hypothesis assumes that breeding groups in TSD species are smaller and more isolated than in GSD species (Burke 1993). However, no data support the derived expectations of higher inbreeding and lower heterozygosity in TSD species than in GSD taxa, or negative correlation of heterozygosity with female-biased sex ratio (Burke 1993). Further, *Alligator mississipiensis*, which might exhibit such a correlation (Burke 1993), may actually not have general female biases (Thorbjarnarson 1997).

Neutral Hypotheses

1. Expression of a preexisting thermal sensitivity

Bull (1981) described the neutral transition from a heterogametic XX/XY GSD system to a TSD system, when an environmental change, or a shift in the species-realized niche, exposes individuals to conditions permitting the expression of a ubiquitously preexisting thermosensitivity. In a species possessing an XX-XY GSD system (with homomorphic or even heteromorphic sex chromosomes), some XX females develop into males in certain thermal environments (Bull 1983, 1989). Because population sex ratio evolves towards ½, the introduction of XX males induces a compensatory reduction in XY-male frequency according to the level of environmental influence *(k),* such that if $k \geq ½$ of the XX individuals develop as males, XY genotypes disappear from the population (Bull 1983). However, if $k < ½$ of the XX individuals become males, there is a continuum of attainable neutral equilibria from pure GSD (XX = XY = ½ when k = 0) to pure ESD (XX = 1 when $k = ½$) if all genotypes within a sex are equally fit—that is, if sex chromosomes are not highly heteromorphic (Bull 1981, 1983, 1989). The same will happen if female heterogamety (ZZ/ZW) is the starting condition. Because thermosensitivity was ubiquitous as the initial condition, this scenario leaves unexplained how this sensitivity first originated and spread.

2. Invasion by a sex ratio distorter

However, TSD can arise as a relatively neutral change or as sex ratio distorter and can invade a GSD population with

differentiated sex chromosomes (male or female heterogamety with lethal YY or WW) (Morjan 2002). Based on *Chrysemys picta*'s life history, Morjan (2002) modeled nest temperatures as a function of female nest-site choice (heritable, nonheritable, or natal philopatry) and climatic conditions. TSD originated as a dominant or recessive mutation in the mitochondria, either sex chromosome, or an autosome. TSD eliminated the W or Y chromosome when TSD (1) was inherited mitochondrially, (2) biased sex ratios towards the heterogametic sex, (3) was dominant to Y or W, or (4) in two isolated simulations of natal philopatry (Morjan 2002). The analytical model showed that when the environment produces $k \geq ½$ females, TSD always invades if transmitted perfectly as a mitochondrial mutation, and if $k = ½$, TSD fully replaces a ZW system. In many other cases, TSD reached intermediate frequencies (Morjan 2002).

The models by Bull (1981, 1983, 1989) and Morjan (2002) demonstrate that positive adaptive selection is not essential to explain TSD origin in vertebrates, although selection (see Adaptive Hypotheses, above) increases the likelihood and rate of its spread and fixation. If Y (or W) chromosomes degenerate (e.g., through heteromorphism) and disappear during GSD evolution (Graves 2001, 2002), the moment of loss constitutes an opportunity for TSD to be quickly established, particularly if TSD works just as well as GSD. Testing of this prediction is required.

TSD Maintenance

Adaptive Hypotheses

The same adaptive hypotheses proposed for the origin of TSD (see TSD Origin, Adaptive Hypotheses, above) could also explain its maintenance, but other adaptive hypotheses only apply to TSD persistence.

1. DIFFERENTIAL FITNESS SUBHYPOTHESIS RELATED TO MATERNAL EFFECTS BY FEMALE NEST-SITE CHOICE

Maternal effects on egg allocation. Purportedly, TSD persists adaptively in some systems where maternal effects on egg allocation prevent the evolution of GSD. Based on data from *Malaclemys terrapin*, Roosenburg (1996) proposed that females choose nesting sites according to the size of their eggs, because egg size is positively correlated with offspring size and translates into differential fitness for sons and daughters (sensu Charnov and Bull 1977). Female hatchlings are postulated to benefit more from a larger initial size through posthatching growth (thus related to the sexual size dimorphism hypothesis). Incubation temperature would allow females to bias offspring sex ratio accordingly (Roosenburg 1996). The model is inapplicable to some turtles. In *Chrysemys picta,* egg mass is positively correlated with hatchling mass (Janzen and Morjan 2002), and larger females attain higher fecundity while smaller males attain higher mating success (Janzen and Morjan 2002 and references therein), yet egg size is uncorrelated with offspring sex (Janzen and Morjan, unpubl. data). Likewise, *Podocnemis expansa* has larger adult females than males. Contrary to expectation, however, larger females likely produce more males than small females because they lay more and larger eggs in deeper (colder) nests (Valenzuela 2001a,b). Further, larger hatchlings grow less than smaller ones, and thermal clines within nests yield mixed sex ratios (Valenzuela 2001a,b), reducing the required maternal sex ratio control. Alternatively to matching sex to egg size via nest-site selection, such matching could occur via allocation of yolk hormones (Bowden et al. 2000), though this requires direct testing. *Macroclemmys temiinckii* merits further investigation in this regard due to a positive correlation between intraclutch egg mass and sex ratio (Ewert et al. 1994). Therefore, maternal allocation of yolk hormones may interact with egg size (Bowden et al. 2000), maternal age (Ewert et al. 1994), and nest-site choice synergistically or antagonistically, such that the effects postulated by Roosenburg (1996) hypothesis may be masked by or be the indirect result of hormones or other factors.

2. ADAPTIVE HYPOTHESES RELATED TO SELECTION FOR BIASED SEX RATIOS

Population stability under high mortality schedules. Based on *Alligator mississippiensis*, Woodward and Murray (1993) proposed that TSD is favored over GSD in crocodilians by allowing adult female biases, which provide population stability under high mortality schedules. The model assumed that (1) female alligators nest preferentially in wet marshes, but if sites are unavailable they nest in dry marshes, and lastly in dry leeves; (2) incubation temperatures in these sites are low, intermediate, and high, and (3) these incubation sites/temperatures produce 100% females, mixed sexes, and 100% males, respectively. However, sex ratios vary annually within habitats due to climatic variation (Rhodes and Lang 1995, 1996), and widespread adult female-biased sex ratios in *A. mississippiensis* and other crocodilians have been contested (Thorbjarnarson 1997; Lance et al. 2000). Additionally, introducing modifier alleles of the individual temperature sensitivity or female nesting behavior triggers the invasion by masculinizing alleles and population extinction, such that the model may not explain the occurrence of TSD in crocodilians (Girondot and Pieau 1996). Finally, use of

cohort or population sex ratios as the selected values in sex ratio evolution models for species with overlapping generations may be inappropriate (Girondot and Pieau 1996).

Sib avoidance. The sib-avoidance hypothesis suggests that TSD could be favored if it reduces the likelihood of inbreeding by producing unisexual clutches (Ewert and Nelson 1991; Burke 1993). Inbreeding avoidance would be important in species with likely sib mating due to geographic fidelity in reproduction or whole clutch mortality from abiotic factors (Ewert and Nelson 1991). Sib avoidance does not explain TSD origin because the advantages arise subsequent to strong within-clutch sex ratio biases (Ewert and Nelson 1991). Further, this hypothesis relies on the unsupported implication that inbreeding should be generally more common in GSD than in TSD species (Burke 1993). It also seems an unlikely explanation for TSD persistence for several reasons: (1) Thermoclines within nests reduce unisex clutch production (Wilhoft et al 1983; Georges 1992; Hanson et al. 1998; Kaska et al 1998; Valenzuela 2001b), (2) TSD cannot prevent cohorts from interbreeding in long-lived/iteroparous taxa (many TSD turtles and crocodilians) (Burke 1993), and (3) multiple paternity reduces inbreeding equally in TSD and GSD taxa (e.g., Harry and Briscoe 1988; Galbraith et al. 1993; Olsson et al. 1994; Hoggren and Tegelstrom 1995; Kichler et al. 1999; Abell 1997; Gullberg et al. 1997; Valenzuela 2000; Davis et al. 2002; Morrison et al. 2002). Therefore, TSD is not a crucial or unique inbreeding avoidance mechanism in many reptiles. Note that the sib-avoidance and group-structured adaptation hypotheses (see TSD Origin, Adaptive Hypotheses, Group-Structured Adaptation) attempt to explain sex ratio biases rather than being an argument for TSD specifically (Bull and Charnov 1988; Burke 1993).

Cultural inheritance. Natal homing through cultural inheritance was suggested to favor skewed primary sex ratios as reported for TSD reptiles (Freedberg and Wade 2001). As defined, natal homing cannot be *culturally* transmitted from mother to daughters in taxa showing no contact after oviposition; it is better explained by imprinting (Cury 1994), without impacting the model. Although some sea turtle data support this hypothesis (Freedberg and Wade 2001), its applicability is restricted for several reasons: (1) Reported sex ratio biases are not general (Gibbons 1990; Mrosovsky 1994; Thorbjarnarson 1997; Girondot et al., Chapter 15), and some correspond to secondary or adult sex ratio skews unrelated to TSD (Gibbons 1990; Mrosovsky 1994). (2) The assumption of temporal stability in sex ratios (as by Reinhold 1998) is inconsistent with observed climatically induced annual sex ratio variation (e.g., Godfrey et al. 1996; Valenzuela and Janzen 2001). (3) Vegetation cover was regarded as the ultimate determinant of turtle sex ratios, but nesting sites for many species lack vegetation. (5) Finally, Freedberg and Wade (2001) concluded that the runaway female bias via natal homing is countered only by genes reducing natal homing (still maintaining TSD), but it actually favors GSD evolution (Bull 1980; Bull and Charnov 1988). Alternatively, persistent sex ratio biases in TSD taxa could be explained by low effective heritabilities of both threshold temperatures and maternal nest-site choice (Bull et al. 1982a, 1988; Rhen and Lang 1995, 1998; Janzen 1992; Olsson et al. 1996).

Neutral Hypotheses

1. TSD-GSD equivalence

The simplest explanation for TSD persistence is that TSD is neutral (Bull 1980; Mrosovsky 1980)—that is, if TSD is as good as GSD, there is no intrinsic selective pressure to evolve GSD. Such is the case under TSD if nest temperatures are uncorrelated with male and female fitness, and under a relatively constant environment inducing a sex ratio equilibrium around ½, (Bull 1980).

2. Phylogenetic inertia

The phylogenetic inertia hypothesis states that TSD is vestigial, maintained because lack of genetic variation prevents GSD from evolving (Bull 1980). This hypothesis does not explain TSD origin because it leaves unexplained how TSD invaded the ancestral species, and it is not entirely neutral because such genetic constraints allow TSD persistence even if maladaptive. The distribution of sex-determining mechanisms among taxa supports multiple independent origins of TSD and GSD in vertebrates, invalidating phylogenetic inertia as a general cause for TSD maintenance (Burke 1993; Janzen and Paukstis 1991b; Chapters 2–7 and 13). However, if all crocodilians do possess TSD, perhaps it is maintained by phylogenetic inertia in this group.

3. Neutralizing effect of life histories and genetic variation

Certain life histories can transform TSD from maladaptive into a neutral or nearly neutral trait. For example, *overlapping generations* counter annual sex ratio fluctuations because multiple cohorts interbreed (Mrosovsky 1994; Girondot and Pieau 1996), an effect enhanced by *longevity* as more cohorts interbreed in a long-lived than in a short-lived species. Additionally, *genetic variation for individual thermal responsiveness* may counter biased sex ratios if variants ensure male and female production under climatic change. It may also permit TSD adjustments through the evolution of al-

lelic frequencies according to environmental changes. However, such evolution may be precluded if climate changes faster than the rate of genetic evolution.

Interestingly, TSD appeared neutral when GSD was rarely known in turtles and unknown in crocodilians (Mrosovsky et al. 1984b), but as reports of GSD turtles increased, adaptive TSD evolution became more plausible (Ewert and Nelson 1991). Currently, support for adaptive explanations of TSD persistence among reptiles remains inconclusive, and neutrality has regained momentum (Girondot and Pieau 1999). Neutrality should be used as the null hypothesis, and falsified directly using adaptiveness as the alternative hypothesis. Perhaps it will be concluded that neutrality often explains TSD persistence satisfactorily, particularly in many long-lived species. Rather, we should ask how large any advantage or disadvantage must be to overcome the neutralizing effect of longevity and overlapping generations.

Only long-term research of incubation temperature effects on lifetime reproductive success will provide conclusive evidence against the neutral hypothesis and in favor of an adaptive explanation. Temperature effects for more than one year remain understudied (Elphick and Shine 1999), and reports of delayed thermal effects are restricted to short-lived, fast-maturing species (e.g., Crews et al 1998). Given the potential prevalence of substantial maternal effects, at least two generations must be reared in common environments to obtain a reasonable assessment of the genetic basis of threshold traits such as TSD (Roff 1996)—difficult for long-lived taxa.

Evolution of TSD Modes: TSD Ia, Ib, or II

Once a TSD system (as any other polyphenic developmental system) is established, the developmental switch regulation must adjust under environmental changes to preserve maximal accuracy of the phenotypic-environment matching. For instance, temperature thresholds and the timing of the thermosensitive period (TSP) could evolve, otherwise cues become unreliable, and if alternative cues are lacking, GSD evolution is favored.

In TSD II, low and high temperatures produce females, while intermediate temperatures produce males. In TSD Ia, low temperatures produce males and high values produce females, while the opposite is true for TSD Ib. TSD II may be the ancestral condition from which TSD Ia and TSD Ib evolved, by species shifting their responses along the temperature range and by some extreme temperature becoming lethal (Deeming and Ferguson 1988; Pieau 1996; Valenzuela 2001b). Alternatively, TSD II may be the transitional stage in the evolution between TSD Ib and TSD Ia. Unfortunately, data on TSD modes is incomplete or inconclusive, partly because some taxa remain unexamined, and partly because some TSD II cases may be experimental artifacts. For example, some TSD II reports derive from laboratory experiments using low temperatures only during the TSP because extended exposure is lethal (Ewert and Nelson 1991; Ewert et al. 1994). Such species with precluded production of low-temperature females are functionally TSD Ia (Valenzuela 2001b), as might occur in *Podocnemis expansa* (Valenzuela 2001b), *P. erythrocephala* (Vogt, unpubl. data), and *Kinosternon leucostomum* (Ewert et al. 1994). Perhaps this inherent TSD II mode reflects (not mutually exclusively) (1) an evolutionary vestigial and ecologically irrelevant ability (since it is unrealized under typical field conditions), or (2) a coaptable faculty for TSD evolution under climate change. Testing the validity of 1 requires sound phylogenetic hypotheses and reliable data on TSD modes. Additionally, it is uncertain whether among-species variation in TSD modes reflects drift or local adaptation, or could derive from the evolution of correlated traits.

TSD and GSD are frequently treated as two single traits, but this is not always appropriate. For instance, since gonadal differentiation can become thermosensitive by modifications at various steps, the independent origins of TSD (Janzen and Pausktis 1991b; Janzen and Krenz, Chapter 13) could generate differing mechanisms molecularly that constitute distinct traits. Similarly, differentiation within taxa sharing a common TSD ancestor could produce distinct TSD mechanisms, although related by the ancestral state. This is important because to understand TSD and GSD evolution we must define and identify the traits unambiguously (Valenzuela et al. 2003).

The Issue of Maternal Effects

A female contributes zygotic genes to her offspring and additional clutch effects through energy and yolk steroids allocation to the egg (Bowden et al. 2000; Elf et al. 2002), maternal-effect genes (maternal mRNA) (Werren et al. 2002), nest-site choice, and perhaps mate choice, since paternal genetic contributions can affect sex ratios in TSD species (Conover and Heins 1987a; Saillant et al. 2002) (Figure 14.1). Nest-site choice directly influences the incubation conditions of the offspring and their subsequent phenotype and fitness, but need not imply active female manipulation of offspring sex as is sometimes assumed explicitly or implicitly (e.g., Roosenburg 1996; Roosenburg and Niewiarowski 1998; Janzen and Morjan 2001, 2002). Similarly, yolk steroid concentrations can affect sex ratios substantially, not necessarily reflecting active sex ratio manipulation via hormonal

allocation (Roosenburg and Niewiarowski 1998), as they could be [passive] by-products of the female's reproductive cycle (Bowden et al. 2000). Caution is necessary because extrinsic factors can skew sex ratios in seeming agreement with deterministically adaptive maternal manipulation if not tested properly (Post et al. 1999).

Nonetheless, parental patch choice theoretically elicits TSD evolution (sensu Charnov and Bull 1977) faster than does increasing the embryonic response to temperature (Bull 1983), if females choose the patch type that confers the highest fitness for each sex in response to environmental influences on their own condition, thus affecting offspring fitness differentially (sensu Trivers and Willard 1973; Roosenburg 1996). Consequently, population sex ratio approaches ½ under TSD, whereas half the offspring of GSD females encounter a disadvantageous patch (Bull 1983). Conversely, the rate of sex ratio evolution can be half as fast via maternal control than by evolution of embryonic responsiveness to temperature, even if the associated heritability is low (Bulmer and Bull 1982; Morjan 2004), such that the impact of maternal choice on thermal incubation conditions for sex ratio evolution (Bull 1983; Janzen and Morjan 2002) might not be as significant. For other scenarios (e.g., Reinhold 1998), under free patch choice, individuals will select the patch conferring the highest fitness, thus eliminating any TSD advantage.

Whether variation in nest-site choice and hormonal allocation (among females, seasonally within females, and among years) are subject to selection, particularly for sex ratio evolution, are entirely separate questions worthy of study. One caveat is that ideally, studies concerned with nest-site choice in TSD evolution should encompass undisturbed populations, since choices in natural versus disturbed habitats can differ dramatically (Hanson et al. 1998; Kolbe and Janzen 2002). Consequently, interpreting data from disturbed habitats (e.g., Woodward and Murray 1993; Janzen and Morjan 2001) requires caution to avoid misleading conclusions about the significance (adaptive or not) of individual behavior. Nevertheless, comparing disturbed and undisturbed populations can reveal factors currently affecting female nesting or population sex ratios, which could impact future population survival.

The Issue of Heritability

Adaptive TSD and sex ratio evolution require the existence of genetic variability, genetic effects, and genotype-by-environment interactions (Rhen and Lang 1998 and references therein). Few estimates of heritability *sensu stricto* of individual thermal responsiveness in reptiles exist (Bull et al. 1982a; Rhen and Lang 1995, 1998; Janzen 1992), and they are most likely inflated by confounding clutch effect components (Bull and Charnov 1988) including dominant and epistatic genetic variance (Conover and Heins 1987b; Olsson et al. 1996; Saillant et al. 2002), maternal effects (Bowden et al. 2000), and environmental (nest) effects (Shine et al. 1997a). Moreover, effective heritabilities are much lower under fluctuating (field) temperature conditions (Bull et al. 1982a), and probably become negligible after correcting for the effect of yolk steroids (see Bowden et al. 2000). Thus, additive genetic variance may be of reduced magnitude, overriden by temperature except around the pivotal temperature, and by epigenetic maternal effects around the pivotal temperature. Consequently, TSD evolution may be constrained in different taxa (but see Rhen and Lang 1998) by lack of genotype-by-temperature interaction (e.g., Janzen 1992), by nest temperature variance (Bull et al. 1982a), or by maternal effects (Bowden et al. 2000). Nonetheless, clutch identity affects reptilian phenotypes (Bull et al. 1982a; Brooks et al. 1991; Van Damme et al 1992; Janzen 1993; Allsteadt and Lang 1995a; Shine and Harlow 1996; Shine et al. 1997a,b), and components other than additive genetic variance can substantially affect sex determination.

TSD Loss: From TSD to GSD

Adaptive Hypotheses

1. INVASION OF TSD BY GSD

TSD can also be invaded by GSD even in the absence of fitness differences within each sex (Bull 1981, 1983). In a TSD species lacking sex chromosomes, a dominant factor *G* may appear, such that *G* carriers are always males while *gg* individuals exhibit TSD. Factor *G* can invade if the proportion of TSD males differs from ½ between generations, a likely occurrence due to environmental variation. Male frequency increases with *G*'s appearance, favoring *gg* individuals to become females, such that male heterogamety (GSD) is established. The same happens if *G* carriers are females. GSD also spreads under spatial rather than temporal environmental variation, with limited gene flow among patches (Bull 1983). Likewise, if the environment is constant but *Gg* males are more fit than *gg* males, GSD is favored (Bull 1981).

2. CONDITIONS THAT RENDER TSD DISADVANTAGEOUS OVER GSD

Sex ratio fluctuations. TSD species can suffer sex ratio fluctuations induced by climatic variation within and among years. Short-lived species, and taxa with nonoverlapping generations are more susceptible to this effect (see TSD Maintenance, Neutral Hypotheses, Neutralizing Effect of Life Histories, above). Such fluctuations partially explain the

adaptive presence of GSD in northernmost populations of *Menidia menidia* (Conover and Heins 1987b).

Production of intersexes. Production of intersexes is another potential cost of TSD (Bull 1981). However, intersexes in TSD vertebrates are rare in nature (Bull 1983; Crews et al. 1994; but see Pieau et al. 1999b), perhaps because canalization of vertebrate sex differentiation prevents their development, at least naturally (but see Crews and Bergeron 1994; Wibbels and Crews 1995; Chardard and Dournon 1999). Further, early-life intersexuality occurs at similar levels and timing in TSD and GSD reptiles (Ewert and Nelson 1991). In species with potentially true intesexes (Pieau et al. 1999a; Pieau 1982), intersexuality is also transient, and adult reproduction is unaffected (Girondot et al. 1998; Pieau et al 1998).

Allee effect. Berec et al. (2001) postulated that at low population sizes or densities, the reduction in individual fitness (Allee effect) narrows the viable temperature ranges for population survival, but the "spatially homogeneous model," as applied to TSD turtles, depends on initial conditions that do not resemble turtle life histories.

Delayed onset or limited sexual dimorphism early in life. Compared to GSD, TSD embryos may suffer delayed onset or limited sexual dimorphism early in life (Bull 1983). For example, embryonic mammals display sexually dimorphic gene expression, metabolism, and developmental rates even prior to the blastocyst stage (Gutiérrez et al 2000), conceivably because sex chromosomes provide sexual identity from the time of fertilization (Valenzuela et al. 2003). Although TSD embryos could potentially express such early dimorphism at constant single-sex temperatures, predicting their sex at early stages in nature is virtually impossible because temperatures fluctuate and thermal effects are cumulative and are exerted relatively late (middle third of incubation in reptiles, and soon after hatching in fishes). Further, at constant temperatures, *Apalone spinifera* (GSD turtle) shows an accelerated chronology of sexual differentiation compared with TSD turtles (Greenbaum and Carr 2001). Whether these effects reduce fitness of TSD individuals remains untested.

Antagonistic pleiotropy. Antagonistic pleiotropy (i.e., selection for one phenotype imposes negative selection on the opposing phenotype due to genetic correlation) is a cost of phenotypic plasticity difficult to analyze because our understanding of TSD's molecular network is incomplete and because the two alternative phenotypes (males and females) must exist for the species to persist. This field of study deserves further research.

Imperfect phenotype-environment matching. Imperfect phenotype-environment matching caused by a low correlation between cues and selective factors or by constraints in developmental sensitivity hinders the adaptiveness of phenotypic plasticity (Moran 1992). The level of adaptiveness becomes dependent on the combination of the magnitude of the fitness differentials and the relative frequency of the selective environments (Moran 1992). Thus, TSD may not be favored if it costs more than having GSD, or if environments favoring both sexes equally are common. However, the level of phenotype-environment matching and related determinants of TSD adaptiveness remain unstudied in TSD taxa.

Parental control of offspring's sex ratio. Finally, parental control of offspring sex ratio by patch quality via nest-site selection (e.g., Reinhold 1998), favors a 1:1 sex ratio through frequency-dependent selection (Julliard 2000), which, if not attainable under TSD, selects for GSD (Bull 1980).

Neutral Hypotheses

Interestingly, no study has explored the invasion of GSD into a TSD system under neutrality. The model by Bull (1981, 1983) (see TSD Loss, Adaptive Hypotheses, Invasion of TSD by GSD) starts with no fitness differences within each sex, but the male bias induced by the introduction of factor *G* favors *gg* individuals to become females through frequency-dependent selection (Bull 1983), and thus this scenario is not neutral. Whether GSD can invade a TSD system under complete neutrality remains an open question.

Predictions from Theory

A series of testable predictions can be derived from the described theoretical background, which should provide null hypotheses for further empirical and theoretical research (Table 14.3).

Presence of TSD in Fish and Absence in Some Vertebrate Groups

Evidence for TSD reptiles and *Menidia* fish was already examined. In this section, the presence TSD in fish and its absence in other vertebrate groups is briefly discussed.

Fish

TSD is reported in numerous fish (Devlin and Nagahama 2002; see also Conover, Chapter 2), but some cases are GSD systems altered by environmental factors (e.g., thermal sex reversals in the presence of sex chromosomes) rather than TSD *sensu stricto*—that is, permanent sex determination by environmental temperature postfertilization without con-

Table 14.3 Testable Predictions about the Presence/Absence of TSD Among Taxa, Based on the Theoretical Models Described in the Text

Species should not have TSD if:	Species could have TSD if:
1. Variation in environmental temperatures skews population sex ratios drastically at the generation time scale (excessive thermal variability).	1. Sex ratio biases are beneficial, highly correlated with temperature, and are constraint by GSD.
2. Sex ratios are highly biased because there is no variation in the environmental temperature experienced by the offspring during development (insufficient thermal variability).	2. Temperature affects fitness directly or is highly correlated with a factor that affects fitness, differentially for males and females.
3. Heteromorphic sex chromosomes are present (production of YY or WW lethals).	3. Fitness differentials between males and females are large enough, and conferred by environments frequently encountered in nature.
4. Sex determination by temperature is genetically linked to a trait that is selected against.	4. Longevity, overlapping generations, and/or genetic variation for temperature sensitivity exist (buffer against sex ratio fluctuations = TSD neutral).
5. Parents or offspring can control the patch that offspring enter (environmental predictability and sex ratio control induce frequency-dependent selection).	
6. Low matching of phenotype to environment (wrong sex produced at a given temperature)	
7. Temperature and the differential-fitness factor are decoupled (low correlation between cue and selective agent).	
8. Patches that confer differential fitness are uncommon (environments favoring both sexes are common).	

sistent genetic differences among sexes (Valenzuela et al. 2003; see also Conover, Chapter 2). TSD adaptiveness in fish remains understudied because research in the wild is scarce (Conover, Chapter 2). TSD in some fish seems nonadaptive and may persist neutrally via longevity and/or phylogenetic inertia (Strüssmann et al. 1996b) while others cases require further study (Römer and Beisenherz 1996).

Amphibians

GSD is the only mechanism found so far in amphibians (reviewed in Solari 1994; Schmid and Steinlein 2001; Chardard et al., Chapter 7). Thermal sex reversal occurs in some species (see Chardard et al., Chapter 7) but at temperatures not typically encountered in the wild, thus reflecting developmental instability at extreme conditions rather than being ecologically relevant (Valenzuela et al. 2003; Schmidt and Steinlein 2001; Chardard et al., Chapter 7). Such lability however, could be coaptable for the evolution of TSD from GSD, which depends on a heritable susceptibility to develop either sex in response to temperature despite the genetic sex identity (Bull 1983).

Snakes

All snakes examined exhibit GSD (ZZ/ZW), with varying degrees of sex chromosome dimorphism (Solari 1994), but whether GSD is primitive or derived is uncertain because the ancestral state remains equivocal (see Janzen and Krenz, Chapter 13). Janzen and Paukstis (1991b) explained TSD absence in snakes by their shorter lifespan relative to turtles and crocodilians. However, lizards generally have shorter lives than snakes, yet some lizards have TSD. Temperature can distort secondary sex ratios as reported for *Pituophis melanoleucus* and *Nerodia fasciata* (Burger and Zappalorti 1988; Dunlap and Lang 1990; reviewed in Viets et al. 1994), but these were cases of documented or potential thermally induced differential mortality rather than TSD per se. Differential mortality also explains sex ratio biases induced by hydric conditions during incubation in elapid snakes (Reichling and Gutzke 1996). TSD absence in snakes is somewhat surprising since favorable conditions for TSD evolution exist in some cases (e.g., Table 14.2), and environmental factors, including temperature, can skew sex ratios in this group. GSD ubiquity in snakes merits further research.

Other Reptiles

Coexistence of TSD and viviparity appears incompatible because live-bearing parents thermally regulate their offspring's development (Bull 1980), such that embryos might experience insufficient thermal variability (Table 14.3). In fact, almost all reported TSD vertebrates are oviparous. *Eulamprus tympanum* could be an unexpected counterexample

(Robert and Thompson 2001), but its TSD classification requires confirmation (Valenzuela et al. 2003). Likewise, sex chromosomes and TSD *sensu stricto* cannot coexist within an individual, and thermal effects on sex ratios in species with sex chromosomes are explained by several alternative phenomena that do not constitute TSD per se (Valenzuela et al. 2003). Thus, the recently reported co-ocurrence of heteromorphic sex chromosomes and TSD in *Bassiana duperreyi* (Shine et al. 2002) is likely an example of thermal sex reversal such as that found in amphibians. Importantly, unlike in amphibians, sex ratio distortions in *B. duperreyi* occur within natural thermal ranges (Shine et al. 2002) and may be adaptive, sensu Charnov and Bull (1977) (Elphick and Shine 1999; Shine et al. 1995). Thermal sex ratio distortions like these are of great evolutionary significance, may be widespread, and warrant continued research.

Varanus lizards ovoposit inside termite nests where temperatures stay quite constant (de Lisle 1996) and should lack TSD (Table 14.3). Indeed, some species *(V. niloticus, V. varius,* and *V. albigularis)* possess identifiable sex chromosomes (GSD) (de Lisle 1996 and references therein), while others remain unexamined. *Paleosuchus trigonatus*, a TSD crocodilian, also uses termite mounds, but because nests are placed against or on top of the mounds (Magnusson et al. 1985), they experience wider temperature fluctuations than *Varanus* nests, allowing TSD to operate.

Birds and Mammals

Both birds and mammals are homeotherms. Since thermal invariance during development via parental thermoregulation would highly skew sex ratios, precluding TSD (Table 14.3), each group probably evolved or maintained their distinct GSD adaptively: female heterogamety (ZZ/ZW) in birds and male heterogamety (XX/XY) in mammals.

Conclusions

Theoretically, TSD can originate neutrally or adaptively, and once established it can evolve through either path independently of its origin. Conclusive evidence for adaptive TSD evolution is restricted to a few cases in vertebrates—for most TSD taxa the neutral null hypothesis remains unfalsified, particularly for long-lived species with overlapping generations. This is not to say that TSD is never adaptive, but rather that further long-term research is needed to reject either alternative conclusively. Most of the existing hypotheses for TSD evolution are not generalizable theoretically or empirically, perhaps because TSD evolved by different means in various vertebrate groups and thus requires case-specific explanations. Only by continuous observation, hypothesis building, and testing will we disentangle TSD evolution. Comparative approaches that explore TSD's physiological and molecular basis in an ecological context and examine the correlation of TSD/GSD's presence/absence with multiple life-history traits simultaneously are necessary to truly address the origin and persistence of TSD.

Acknowledgments—Many thanks to Claude Pieau, Mike Ewert, Carrie Morjan, Rachel Bowden, and Dean Adams for their insightful criticisms and suggestions on this manuscript.