

9

Temperature-dependent sex determination

N. Valenzuela

Reptiles possess two main mechanisms of sex determination. In some species the sex of the individual is determined at conception by sex factors contained in sex chromosomes (termed genotypic sex determination or GSD). However, in other species sex is determined after conception by the environmental temperature during incubation and cannot be predicted by zygotic genotype (Bull, 1983; Valenzuela *et al.*, 2003). This peculiar mechanism, termed 'temperature-dependent sex determination' (TSD) is one of several existing types of environmental sex determination among animals but the only one so far described reliably for reptiles. TSD has persisted for millions of years although it is still not known whether TSD is ancestral to reptiles. Sex ratios under TSD can be biased and the prevalence of this mechanism over time is somewhat surprising given the drastic environmental changes in temperatures that the earth has undergone. In this chapter how the incubation environment affects sex determination in reptiles from a thermal and molecular standpoint is reviewed, and the evolution of TSD is briefly considered.

Sex determining systems

Sex chromosomes in GSD species can differ morphologically from each other (heteromorphic) or not (homomorphic). In some species exhibiting the XX/XY sex chromosomes males are heterogametic (XY), while in other species with the ZZ/ZW system females are heterogametic (Bull 1983). Some reptiles exhibit heteromorphic sex chromosomes while others possess homomorphic sex chromosomes (reviewed in Solari 1994). Primary sex ratios are usually balanced (1:1) in heterogametic systems due to the Mendelian segregation of sex chromosomes. However, environmental factors may override sex chromosomes and distort primary sex ratios (Valenzuela *et al.*, 2003). Among those are temperature-induced differential mortality as observed in the pine snake *Pituophis melanoleucus* (Burger and Zappalorti, 1988), embryo abortion as reported for the mouse (*Mus musculus domesticus*) and in the viviparous skink, *Chalcides chalcides* (*e.g.* Krackow, 1992; Blackburn *et al.*, 1998), and differential fertilization as described for the bird *Acrocephalus sechellensis* and the cricket *Grillodes*

Temperature-dependent sex determination

supplicans (e.g. Komdeur *et al.*, 1997; Stockley, 1999). Temperature is also known to bias secondary sex ratios by inducing sex reversal in species with sex chromosomes (Dournon *et al.*, 1990; Solari, 1994; Baroiller *et al.*, 1995).

On the other hand, some species do not display sex chromosomes, no consistent genetic differences exist between the sexes, and sex determination occurs after fertilisation by incubation temperature (Bull, 1983; Solari, 1994). In this last system (*i.e.* TSD), primary sex ratios are not defined at conception, but are instead realised after fertilisation according to the incubation temperature, and can often be biased (Valenzuela *et al.*, 2003). A set of criteria to identify the presence of TSD unambiguously has been proposed by Valenzuela *et al.* (2003). A series of detailed reviews about the prevalence, ecology and evolution of TSD in vertebrates can be found in Valenzuela and Lance (2004).

TSD has been observed in all crocodylians (13 of 25 species) and tuataras (both species) so far examined, is prevalent in turtles (67/85 of approximately 245 species have been examined), and is less frequent in lizards (37/161 species examined from approximately 3,750 species). The sex determining mechanism of individual species are shown in Table 9.1. The incidence of TSD differs between Families. Whilst many turtles exhibit TSD to date no example has been reported for the Families Trionychilidae or Chelidae. In lizards, TSD is common in the Agamidae and Gekkonidae but reports are absent in Teiidae, Phrynosomatidae and Polychrotidae.

Table 9.1. Reported cases of sex determining mechanism (SDM) among reptiles (excluding snakes – see text). Only families where either temperature-dependent sex determination (TSD) or genetic sex determination (GSD) has been determined from incubation temperature experiments or the identification of sex chromosomes are included.

<i>Species</i>			<i>Species</i>		
	<i>SDM</i>	<i>Source</i>		<i>SDM</i>	<i>Source</i>
RHYNCOCEPHALIA			Alligatoridae		
<i>Paleosuchus trigonatus</i>	TSD	34	<i>Crocodylus palustris</i>	TSD	32
Sphenodontidae			<i>Alligator mississippiensi</i>	TSD	16
Crocodylidae			<i>Crocodylus porosus</i>	TSD	57
<i>Sphenodon guntheri</i>	TSD	7	<i>Alligator sinensis</i>	TSD	9
<i>Crocodylus johnstoni</i>	TSD	57	<i>Crocodylus siamensis</i>	TSD	31
<i>Sphenodon punctatus</i>	TSD	40	<i>Caiman crocodilus</i>		
<i>Crocodylus moreletii</i>	TSD	31	<i>crocodilus</i>	TSD	31
CROCODYLIA			Gavialidae		
<i>Crocodylus niloticus</i>	TSD	24	<i>Caiman crocodilus yacare</i>	TSD	4

Species	SDM Source	Species	SDM Source
<i>Gavialis gangeticus</i>	TSD	31	<i>Emys [Clemmys]</i>
<i>Caiman latirostris</i>	TSD	61	<i>marmorata</i>
CHELONIA			TSD
Pelomedusidae			14
<i>Pelomedusa subrufa</i>	TSD	13	<i>Graptemys barbouri</i>
<i>Pelusios castaneus</i>	TSD	13	<i>Graptemys geographica</i>
<i>Podocnemis erythrocephala</i>	TSD	55	<i>Graptemys kohnii</i>
<i>Podocnemis expansa</i>	TSD	50	<i>Graptemys nigrinoda</i>
<i>Podocnemis unifilis</i>	TSD	8	<i>Graptemys ouachitensis</i>
Bataguridae			TSD
<i>Chinemys nigricans</i>	TSD	15	<i>Graptemys pseudogeo-</i>
<i>Chinemys reevesi</i>	TSD	33	<i>graphica</i>
<i>Kachuga smithii</i>	GSD	46	<i>Graptemys pulchra</i>
<i>Mauremys mutica</i>	TSD	13	<i>Graptemys versa</i>
<i>Mauremys annamansis</i>	TSD	15	<i>Heosemys grandis</i>
<i>Melanochelys trijuga</i>	TSD	13	<i>Malaclemys terrapin</i>
<i>Rhinoclemmys areolata</i>	TSD	13	<i>Pseudemys concinna</i>
<i>Rhinoclemmys pulcherrima</i>	TSD	13	<i>Pseudemys floridana</i>
<i>Siebenrockiella crassicollis</i>	GSD	5	<i>Pseudemys nelsoni</i>
Carettochelyidae			TSD
<i>Carettochelys insculpta</i>	TSD	56	<i>Pseudemys texana</i>
Cheloniidae			TSD
<i>Caretta caretta</i>	TSD	59	<i>Terrapene carolina</i>
<i>Chelonia mydas</i>	TSD	38	<i>Terrapene ornata</i>
<i>Eretmochelys imbricata</i>	TSD	39	<i>Trachemys scripta</i>
<i>Lepidochelys kempii</i>	TSD	47	<i>Trachemys decorata</i>
<i>Lepidochelys olivacea</i>	TSD	36	Kinosternidae
<i>Natator depressus</i>	TSD	23	<i>Claudius angustatus</i>
Chelydridae			GSD
<i>Chelydra serpentina</i>	TSD	58	54
<i>Macrolemys temminckii</i>	TSD	13	<i>Kinosternum acutum</i>
Dermatemydidae			TSD
<i>Dermatemys mawei</i>	TSD	54	26 ^R
Dermochelyidae			15
<i>Dermochelys coriacea</i>	TSD	45	<i>Kinosternon alamosae</i>
Emydidae			TSD
<i>Chrysemys picta</i>	TSD	13	<i>Kinosternon arizonense</i>
<i>Clemmys guttata</i>	TSD	13	TSD
<i>Clemmys insculpta</i>	GSD	13	<i>Kinosternon baurii</i>
<i>Deirochelys reticularia</i>	TSD	13	TSD
<i>Emydoidea blandingii</i>	TSD	13	<i>Kinosternon creaseri</i>
<i>Emys orbicularis</i>	TSD	44	TSD
			<i>Kinosternon cruentatum</i>
			TSD
			<i>Kinosternon flavescens</i>
			TSD
			<i>Kinosternon hirtipes</i>
			TSD
			<i>Kinosternon leucostomum</i>
			TSD
			<i>Kinosternon scorpioides</i>
			TSD
			<i>Kinosternon sonoriense</i>
			TSD
			<i>Kinosternon subrubrum</i>
			TSD
			<i>Staurotypus salvinii</i>
			GSD
			13
			<i>Staurotypus triporcatus</i>
			GSD
			13
			<i>Sternotherus carinatus</i>
			TSD
			<i>Sternotherus minor</i>
			TSD
			<i>Sternotherus odoratus</i>
			TSD
			Testudinidae
			<i>Geochelone elephantopus</i>
			TSD
			26 ^R

Temperature-dependent sex determination

Species	SDM Source	Species	SDM Source
<i>Geochelone gigantea</i>	TSD 26 ^R	<i>Lophognathus temporalis</i>	TSD 22
<i>Gopherus agassizii</i>	TSD 48	<i>Physignathus lesueurii</i>	TSD 22
<i>Gopherus polyphemus</i>	TSD 10	<i>Pogona barbata</i>	GSD 22
<i>Testudo graeca</i>	TSD 43	<i>Pogona minor</i>	GSD 22
<i>Testudo hermanni</i>	TSD 11	<i>Pogona vitticeps</i>	GSD 22
Trionychidae		<i>Tympanocryptis diemensis</i>	GSD 22
<i>Apalone mutica</i>	GSD 25	<i>Tympanocryptis tetra-</i>	
<i>Apalone spinifera</i>	GSD 1	<i>porophora</i>	GSD 22
<i>Pelodiscus sinensis</i>	GSD 63	Anguidae	
<i>Trionyx muticus</i>	GSD 13	<i>Gerrhonotus multi</i>	
Chelidae		<i>-carinatus</i> ^Q	TSD 22 ^R
<i>Chelodina longicollis</i>	GSD 17	Chamaeleontidae	
<i>Elseya novaguineae</i>	GSD 15	<i>Chamaeleo chamaeleon</i> ^F	TSD 22 ^R
<i>Elusor macrurus</i>	GSD 18	<i>Chamaeleo lateralis</i> ^E	TSD 22 ^R
<i>Emydura macquarii</i>	GSD 49	<i>Chamaeleo pardalis</i>	GSD 53 ^R
<i>Emydura signata</i>	GSD 3	Corytophanidae	
<i>Emydura subglobosa</i>	GSD 15	<i>Basiliscus plumifrons</i> [*]	GSD 53
<i>Mesoclemmys [Phrynops]</i>		Crotaphitidae	
<i>gibbus</i>	GSD 15	<i>Crotaphytus collaris</i> [*]	GSD 53
<i>Phrynops geoffroanus</i>	GSD 15	Diplodactylidae	
<i>Phrynops hilarii</i>	GSD 15	<i>Rhacodactylus auriculatus</i>	TSD 22 ^R
<i>Platemys radiolata</i>	GSD 35	<i>Rhacodactylus chahoua</i>	TSD 22 ^R
SQUAMATA		<i>Rhacodactylus ciliatus</i>	TSD 22 ^R
Agamidae		<i>Rhacodactylus leachianus</i>	TSD 22 ^R
<i>Agama agama</i>	TSD 6	<i>Rhacodactylus sarasinorum</i>	TSD 22 ^R
<i>Agama caucasia</i>	TSD 22 ^R	Eublepharidae	
<i>Agama impalearis</i>	TSD 12	<i>Coleonyx brevis</i>	GSD 53
<i>Agama stellio</i>	TSD 22 ^R	<i>Coleonyx variegatus</i>	GSD 53
<i>Amphibolurus muricatus</i>	TSD 22	<i>Eublepharis macularis</i>	TSD 53
<i>Amphibolurus nobbi</i>	GSD 22	<i>Hemitheconyx caudicinctus</i>	TSD 53
<i>Amphibolurus norrisi</i>	GSD 22	Gekkonidae	
<i>Calotes versicolor</i>	GSD 22 ^R	<i>Gehyra australis</i>	GSD 37 ^R
<i>Chlamydosaurus kingii</i>	TSD 22	<i>Gehyra nana</i>	GSD 37 ^R
<i>Ctenophorus decresii</i>	TSD 22	<i>Gehyra purpurascens</i>	GSD 37 ^R
<i>Ctenophorus fordi</i>	GSD 22	<i>Gekko gekko</i>	GSD 37 ^R
<i>Ctenophorus ornatus</i> [*]	TSD 22	<i>Gekko japonicus</i> ^Q	TSD 52 ^R
<i>Ctenophorus pictus</i>	TSD 22	<i>Heteronotia binoei</i>	GSD 37 ^R
<i>Diporiphora albilabris</i>	GSD 22	<i>Phelsuma cepediana</i> ^T	GSD 53 ^R
<i>Diporiphora bilineata</i>	GSD 22	<i>Phelsuma dubia</i> ^E	TSD 53 ^R
<i>Hypsilurus spinipes</i>	GSD 22	<i>Phelsuma guentheri</i> [*]	TSD 53 ^R
<i>Lophognathus burnsi</i> [*]	TSD 22	<i>Phelsuma guimbeaui</i> ^F	TSD 53
<i>Lophognathus gilberti</i>	TSD 22	<i>Phelsuma lineata prusila</i> ^{*F}	TSD 53 ^R

Species	SDM Source	Species	SDM Source
<i>Phelsuma madagascariensis grandis</i>	TSD 53	<i>Ophisops elegans</i>	GSD 42
<i>Phelsuma m. madagascariensis</i> * ^F	TSD 53 ^R	<i>Pedioplanis namaquensis</i>	GSD 42
<i>Phelsuma ornata</i> ^T	GSD 53 ^R	<i>Podarcis erhardii</i>	GSD 42
<i>Phelsuma sundbergi</i> ^T	GSD 53 ^R	<i>Podarcis melisellensis</i>	GSD 42
<i>Phyllodactylus lanei</i>	GSD 37 ^R	<i>Podarcis muralis</i>	GSD 53 ^R
<i>Phyllodactylus marmoratus</i>	GSD 37 ^R	<i>Podarcis pityusensis</i> *	TSD 22 ^R
<i>Tarentola angustimentalis</i> ^E	TSD 53 ^R	<i>Podarcis sicula</i>	GSD 42
<i>Tarentola annularis</i> ^E	TSD 53 ^R	<i>Podarcis tiliguerta</i>	GSD 42
<i>Tarentola boettgeri hierrensis</i>	TSD 53 ^R	<i>Podarcis wagleriana</i>	GSD 42
<i>Tarentola delalandii</i> ^E	TSD 53 ^R	<i>Psammodromus algirus</i>	GSD 42
<i>Tarentola gomerensis</i> ^E	TSD 53 ^R	<i>Takydromus sexlineatus</i>	GSD 42
<i>Tarentola mauritanica</i>	TSD 53 ^R		
Iguanidae		Phrynosomatidae	
<i>Cupriganus achalensis</i>	GSD 20	<i>Sceloporus chrysostictus</i>	GSD 19 ^R
<i>Dipsosaurus dorsalis</i> ^E	GSD 22 ^R	<i>Sceloporus gadoviae</i>	GSD 19 ^R
<i>Phymaturus palluma</i>	GSD 41 ^R	<i>Sceloporus grammicus</i>	GSD 21
<i>Polychrus marmoratus</i>	GSD 20	<i>Sceloporus jarrovi</i>	GSD 53 ^R
<i>Tropidurus torquatus</i>	GSD 41 ^R	<i>Sceloporus lundelli</i>	GSD 19 ^R
Lacertidae		<i>Sceloporus maculosus</i>	GSD 19 ^R
<i>Acanthodactylus erythrurus</i>	GSD 42	<i>Sceloporus merriami</i>	GSD 19 ^R
<i>Eremias arguta</i>	GSD 42	<i>Sceloporus nelsoni</i>	GSD 19 ^R
<i>Eremias olivieri</i>	GSD 19 ^R	<i>Sceloporus occidentalis</i> *	GSD 53 ^R
<i>Eremias velox</i>	GSD 42	<i>Sceloporus poinsetti</i>	GSD 19 ^R
<i>Gallotia galloti</i>	GSD 42	<i>Sceloporus pyrocephalus</i>	GSD 19 ^R
<i>Heliobus lugubris</i>	GSD 42	<i>Sceloporus scalaris</i>	GSD 41 ^R
<i>Lacerta agilis</i>	GSD 42	<i>Sceloporus undulatus</i>	GSD 53
<i>Lacerta armeniaca</i>	GSD 42	<i>Sceloporus uniformis</i>	GSD 19 ^R
<i>Lacerta dugesii</i>	GSD 42	<i>Uta antiquus</i>	GSD 19 ^R
<i>Lacerta horvathi</i>	GSD 42	<i>Uta nolascensis</i>	GSD 19 ^R
<i>Lacerta kulzeri</i>	GSD 62	<i>Uta palmeri</i>	GSD 19 ^R
<i>Lacerta laevis</i>	GSD 62	<i>Uta squamata</i>	GSD 19 ^R
<i>Lacerta lepida</i>	GSD 42	<i>Uta stansburiana</i>	GSD 19 ^R
<i>Lacerta monticola</i>	GSD 42	<i>Uma inornata</i>	GSD 19 ^R
<i>Lacerta strigata</i>	GSD 27 ^R		
<i>Lacerta trilineata</i>	GSD 19 ^R	Polychrotidae	
<i>Lacerta viridis</i>	GSD 53 ^R	<i>Anolis acutus</i>	GSD 19 ^R
<i>Lacerta vivipara</i>	GSD 30	<i>Anolis bimaculatus</i>	GSD 19 ^R
<i>Meroles cuneirostris</i>	GSD 42	<i>Anolis biporcatus</i>	GSD 19 ^R
		<i>Anolis carolinensis</i>	GSD 53
		<i>Anolis conspersus</i>	GSD 19 ^R
		<i>Anolis cooki</i>	GSD 19 ^R
		Anolis cristatellus	GSD 19 ^R
		<i>Anolis distichus</i>	GSD 19 ^R
		<i>Anolis evermanni</i>	GSD 19 ^R

Temperature-dependent sex determination

<i>Species</i>	<i>SDM Source</i>	<i>Species</i>	<i>SDM Source</i>
<i>Anolis ferreus</i>	GSD 19 ^R	Teiidae	
<i>Anolis gingivinus</i>	GSD 19 ^R	<i>Cnemidophorus inornatus</i>	GSD 53 ^R
<i>Anolis gundlachi</i>	GSD 19 ^R	<i>Cnemidophorus tigris</i>	GSD 19 ^R
<i>Anolis krugi</i>	GSD 19 ^R	<i>Cnemidophorus uniparens</i>	GSD 53 ^R
<i>Anolis leachi</i>	GSD 19 ^R	Scincidae	
<i>Anolis lividus</i>	GSD 19 ^R	<i>Bassiana duperreyi</i> ^Q	G/T 51 ^R
<i>Anolis marmoratus</i>	GSD 19 ^R	<i>Cyclodina olivieri</i>	GSD 41 ^R
<i>Anolis nebulosus</i>	GSD 19 ^R	<i>Eulamprus tympanum</i> ^E	TSD 52 ^R
<i>Anolis oculatus</i>	GSD 19 ^R	<i>Eumeces fasciatus</i>	GSD 53
<i>Anolis opalinus</i>	GSD 19 ^R	<i>Eumeces obsoletus</i>	GSD 53
<i>Anolis poncensis</i>	GSD 19 ^R	<i>Oligosoma suteri</i>	GSD 60
<i>Anolis pulchellus</i>	GSD 19 ^R	<i>Scincella lateralis</i>	GSD 41 ^R
<i>Anolis sabanus</i>	GSD 19 ^R	Varanidae	
<i>Anolis scriptus</i>	GSD 19 ^R	<i>Varanus acanthurus</i>	GSD 41 ^R
<i>Anolis stratulus</i>	GSD 19 ^R	<i>Varanus exanthematicus</i>	
<i>Anolis watsi</i>	GSD 19 ^R	<i>albigularis</i>	GSD 29
<i>Tripidodactylus onca</i> ^T	GSD 19 ^R	<i>Varanus niloticus niloticus</i>	GSD 29
Pygopodidae		<i>Varanus salvator</i> ^{*E}	TSD 22 ^R
<i>Delma inornata</i>	GSD 28	<i>Varanus varius</i>	GSD 29
<i>Lialis burtonis</i>	GSD 19 ^R		

G/T: co-existence of TSD and GSD. ^RReviewed in the cited publication. ^{*}Tentative results due to extremely small sample sizes and/or insufficient temperature treatments. ^QQuestionable TSD classification as species has sex chromosomes. ^FTentative classification: only (or nearly exclusively) females were produced at all temperatures employed. ^EEquivocal classification. ^TTentative classification. References cited may not be the only or first description of the SDM for a given species.

Source: 1: Bull and Vogt (1979); 2: Bull *et al.* (1982); 3: Bull *et al.* (1985); 4: Campos (1993); 5: Carr and Bickman (1981); 6: Charnier (1966); 7: Cree *et al.* (1995); 8: De Souza and Vogt (1994); 9: Deeming (2004b); 10: Demuth (2001); 11: Eendeback (1995); 12: El Mouden *et al.* (2001); 13: Ewert and Nelson (1991); 14: Ewert *et al.* (1994); 15: Ewert *et al.* (2004); 16: Ferguson and Joanen (1982, 1983); 17: Georges (1988); 18: Georges and McInnis (1998); 19: Gorman (1973); 20: Gorman *et al.* (1967); 21: Hall and Selander (1973); 22: Harlow (2004); 23: Hewavisenanthi and Parmenter (2001); 24: Hutton (1987); 25: Janzen (1993); 26: Janzen and Paustkis (1991b); 27: King (1977); 28: King (1990); 29: King and King (1975); 30: Kupriyanova (1990); 31: Lang and Andrews (1994); 32: Lang *et al.* (1989); 33: Ling (1985); 34: Magnusson *et al.* (1990); 35: McBee *et al.* (1985); 36: McCoy *et al.* (1983); 37: Moritz (1990); 38: Mrosovsky *et al.* (1984); 39: Mrosovsky *et al.* (1992); 40: Nelson *et al.* (2004); 41: Olmo (1986); 42: Olmo *et al.* (1990); 43: Pieau (1971); 44: Pieau (1974); 45: Rimblot *et al.* (1985); 46: Sharma *et al.* (1975); 47: Shaver *et al.* (1988); 48: Spotila *et al.* (1994); 49: Thompson (1983); 50: Valenzuela (2001); 51: Valenzuela (2004); 52: Valenzuela *et al.* (2003); 53: Viets *et al.*

N. Valenzuela

(1994); 54: Vogt and Flores-Villela (1992); 55: Vogt, unpublished data; 56: Webb *et al.* (1986); 57: Webb *et al.* (1987c); 58: Yntema (1976); 59: Yntema and Mrosovsky (1979); 60: Hare *et al.* (2002); 61: Piña *et al.* (2003); 62: den Bosch *et al.* (2003); 63: Ji *et al.* (2003).

All snakes examined to date exhibit GSD with a ZZ/ZW female-heterogamety system with varying degrees of sex-chromosome dimorphism (Solari 1994). The apparent lack of TSD in snakes is noteworthy because conditions purportedly favourable for TSD evolution exist in some species (Burger and Zappalorti, 1988; Webb *et al.*, 2001; reviewed in Valenzuela 2004). Janzen and Paukstis (1991a) proposed that TSD absence in snakes might be due to their shorter lifespan relative to turtles and crocodylians. However, lizards generally live shorter than snakes, yet some have TSD. The ubiquity of GSD in snakes should be further examined.

Thermal mechanism

Three TSD patterns are defined by the sex ratios produced as a function of incubation temperature, all of which are present in reptiles (Figure 9.1). In TSDII (also termed female-male-female or FMF in the literature), females are produced at low and high temperatures and males at intermediate temperatures. In TSDIa (also termed male-female or MF), males are produced at low temperatures and females at high temperatures, while the opposite is true for TSDIb (also termed female-male or FM). Results from constant incubation conditions are used to elucidate the pattern that describes the TSD mode for a given species. Another important parameter is the pivotal (also called threshold) temperature, which is the constant incubation temperature that produces a population-wide 1:1 sex ratio. TSDII systems have two (a lower and higher) pivotal temperatures, whereas TSDIa and TSDIb have a single pivotal temperature (Figure 9.1). The range of temperatures that produces mixed sex ratios is termed the transitional range (TR). A single transitional range characterises TSDIa and TSDIb, while TSDII exhibits two (Figure 9.1). TSDII has been reported for turtles, lizards, and crocodylians; TSDIa for turtles; and TSDIb for tuataras, lizards and crocodylians (see reviews in Valenzuela and Lance, 2004). The temperatures that sustain development and produce the entire array of sex ratios vary among TSD species as well as among population within a TSD species. Variation is also found in the pivotal temperature, and in the width, slope and symmetry of the transitional range.

Temperature determines sex during a limited window of developmental time called the thermosensitive period (TSP) which can be established from the results of transfer experiments, where embryos start incubation at a temperature that produces a single sex and groups of embryos are shifted at sequential times during

Temperature-dependent sex determination

development to the other extreme temperature that produces exclusively the opposite sex (Figure 9.2). A sex ratio equal to that expected by the second temperature indicates that the TSP had not started by the transfer time, whereas a sex ratio equal to that expected by the first temperature indicates that the TSP had ended by the transfer time. Intermediate sex ratios to those expected by either temperature suggest an active TSP at the time of transfer.

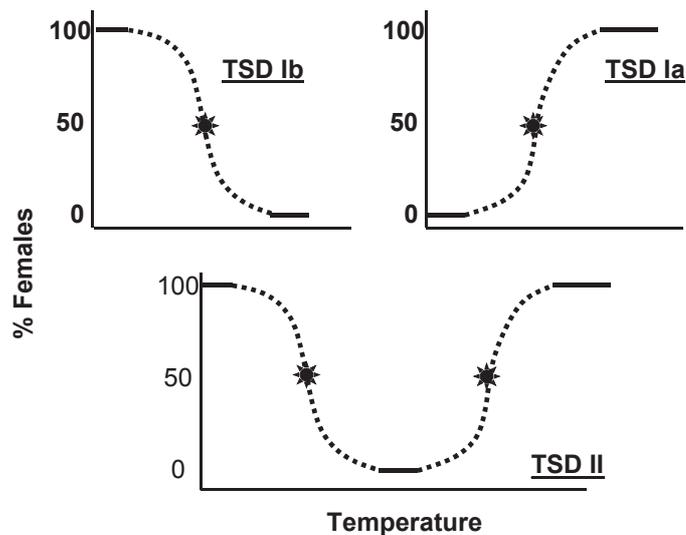


Figure 9.1. The three patterns of temperature-dependent sex determination (TSDIa, TSDIb, TSDII) defined by the sex ratios produced as a function of constant incubation temperature. Dotted lines represent the transitional range(s). Stars denote the pivotal temperatures that correspond to constant temperatures that produce a population-wide 1:1 sex ratio. Modified from Valenzuela (2004).

Once the influence of temperature on sex ratios was demonstrated in laboratory studies, researchers tested the biological significance of their results by trying to reproduce them in nature. Some studies on crocodylians and sea turtles conducted in the field found that mean incubation temperature predicted sex ratios, in accordance with the mean constant temperature used in laboratory conditions for each species (*e.g.* Ferguson and Joanen, 1982, 1983; Morreale *et al.*, 1982; Mrosovsky *et al.*, 1984, 1992; Standora and Spotila, 1985; Magnusson *et al.*, 1985, 1990; Spotila *et al.*, 1987; Rhodes and Lang, 1996). However, results from constant-temperature incubation experiments may not mimic what occurs under field conditions where incubation temperature varies daily (Chapter 2), sometimes drastically (see lower panel of Figure 9.2). In other reptiles, differences between lab and field results were found both in terms of development (*e.g.* timing of the TSP) and sex ratio production as illustrated in Figure 9.2 (*e.g.* Pieau, 1982; Wilhoft

N. Valenzuela

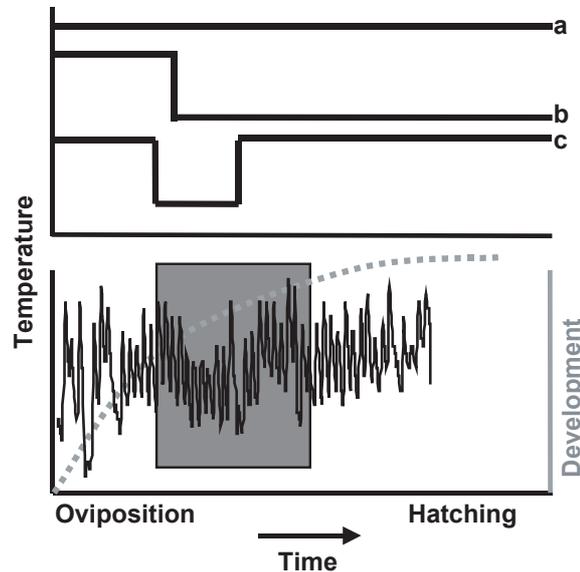


Figure 9.2. Line 'a' in the upper panel represents a constant temperature incubation experiment; line 'b' represents a transfer-once experiment from a high temperature to a low temperature; and line 'c' represents a transfer-twice experiment starting at high temperature then shifting to a low temperature and returning to the initial high temperature. The lower panel depicts the incubation temperatures (solid line) recorded from a field nest (data from Valenzuela 2001b), a hypothetical developmental curve as developmental stages reached over time (dotted line), and the thermosensitive period (indicated by grey area in box). Temperature fluctuations affect developmental rate (changing the slope of the dotted line), such that changes in development may retard or speed up the onset and length of the TSP in absolute time (sliding the grey window along the time axis) in comparison to the absolute TSP timing as defined by transfer experiments using constant incubation temperatures. Modified from Valenzuela (2004).

et al., 1983; Bull, 1985; Schwarzkopf and Brooks, 1985; Georges, 1989, 1992; Mrosovsky and Provancha, 1992; Campos 1993; De Souza and Vogt, 1994; Pinheiro *et al.*, 1997; Valenzuela *et al.*, 1997; Harlow and Taylor, 2000; Valenzuela, 2001; Deeming, 2004b). In fact, in some field studies a combination of mean and variance was required to explained sex ratios, suggesting that temperature fluctuation also had an effect (*e.g.* Bull, 1985; De Souza and Vogt, 1994). These two parameters did not suffice in other studies and new models were developed to account for the results (Pieau, 1982; Schwarzkopf and Brooks, 1985; Georges, 1989, 1992; Valenzuela, 2001). Laboratory studies using controlled fluctuating temperatures that generate identical cycles of temperature (thus keeping a constant variance) were implemented in order to simulate more natural conditions or to test the effect of factors other than temperature on sex ratio (*e.g.* Bull and Vogt 1979; Paukstis *et al.*, 1984). The number of hours at or above a pivotal temperature during the critical period was found to be good predictor of sex ratios in several cases (*e.g.* Pieau, 1982; Wilhoft *et al.*, 1983; Bull, 1985; Schwarzkopf and Brooks, 1985; Mrosovsky and Provancha, 1992; De Souza and Vogt, 1994). The results of

Temperature-dependent sex determination

those studies are consistent with laboratory results at a qualitative level (Bull and Vogt, 1979; Pieau, 1974, 1982; Wilhoft *et al.*, 1983; Vogt and Bull, 1984; Georges, 1992) and more rarely at the quantitative level (see Bull, 1985). Laboratory-field comparisons are almost non-existent for lizards because nest temperatures have not been studied extensively in this group (Harlow and Taylor, 2000). Small-clutch sizes also precludes statistical comparisons in some species (*e.g.* Harlow and Taylor, 2000)

Georges (1989) developed a mathematical model to analyse data on fluctuating incubation temperatures using the amount of daily development that occurs above or below the pivotal temperature. The predictor of sex in this case is termed the 'Constant Temperature Equivalent', *i.e.* the temperature above and below which half of development occurs (Georges *et al.*, 1994). An improved algorithm for this model is presented in Georges *et al.* (2003). An alternative but related statistical model was developed by Valenzuela (2001) to account for the effects of heterogeneous daily fluctuations of natural nest temperatures. This showed that a combination of 'Cumulative Temperature Units' (sum of degree-hours cut off at the low constant temperature survival threshold) and time spend below the low constant temperature survival threshold explained significant variation in sex ratio when laboratory and field data were combined. Approaches like these, that take into account the effect of both duration and magnitude of the incubation temperature on development and sex ratio seem to be the most promising as predictors of sex in natural settings (see Nelson *et al.*, 2004). They may also allow a general model to be developed that encompasses the highest number of species. Eventually, it should be possible to tell whether TSD is different between species, as the variety of results from field studies may imply, or if there are common basis to this mechanism in all cases.

Molecular underpinnings

Important advances have been made in our knowledge of the molecular mechanism of genotypic sex determination (reviewed in Sinclair, 1998; Hayes, 1998; Clinton, 1998; Graves, 1998; Pelliniemi, 1998; Jerasuria and Place, 1998; Pieau *et al.*, 1999; Morrish and Sinclair, 2002), although our understanding remains incomplete (Bowles *et al.*, 2000; Ballejos *et al.*, 2001). Common genes to TSD and GSD (reptilian homologues of mammalian and avian genes) that regulate the gonadal differentiation cascade have been identified (*e.g.* SOX9, SF1, WT1, DAX1, DMRT1, AMH, and Aromatase). However, the factor(s) that renders TSD systems unique (*i.e.* susceptible to temperature) remains undescribed (see also the review by Place and Lance, 2004). Irrespective of whether or not any of these identified sex-factors is the ultimate controlling locus that causes the initial commitment of

N. Valenzuela

the embryo to the female or male pathways, or whether their differential expression is the consequence of as yet unidentified TSD-genes whose action occurs earlier, their influence on sex differentiation is well documented under laboratory conditions. Here what is known about these molecular elements in TSD reptiles is briefly reviewed.

Recent research on reptilian TSD has partially deciphered some of the molecular bases and physiological pathways of sex differentiation (*e.g.* Johnston *et al.*, 1995; Jeyasuria *et al.*, 1995; Crews, 1996; Pieau, 1996; Conley *et al.*, 1997; Lance, 1997; Janzen *et al.*, 1998; Pieau *et al.*, 1998; Spotila *et al.*, 1998; Wibbels *et al.*, 1998; Bowden *et al.*, 2000, 2001). Molecular studies on TSD have identified and analysed common elements between GSD and TSD species, and have found that some of those elements in TSD species have temperature-related differential expression concordant to their expression in GSD by sex and described below.

Additionally, researchers have shown that in some TSD species there is a synergistic influence of oestrogens and temperature (Gutzke and Chymiy, 1988; Crews *et al.*, 1989; Dorizzi *et al.*, 1991; Wibbels *et al.*, 1991; Crews, 1996). Crews (1996) suggested that sex steroid hormones serve as the proximate trigger for male and female sex determination and that they are the physiological equivalent of incubation temperature for sex determination. Aromatase, the enzyme responsible for the aromatisation of androgens to oestrogens, plays a key role in the feminisation of turtle gonads (Desvages and Pieau, 1992b; Jeyasuria *et al.*, 1994), and its level of expression is more important for ovarian development than simply the presence/absence of expression (Place *et al.*, 2001). Further, the activity of aromatase or its synthesis can be temperature sensitive (Desvages and Pieau, 1992a, 1992b). Aromatase activity is higher at female- than at male-producing temperatures, and intermediate at the pivotal temperature (Pieau *et al.*, 1999). However, putative female embryos at the pivotal temperature still exhibit higher aromatase activity than male embryos (Pieau *et al.*, 1999). Recent evidence suggests that aromatase competes with 5-alpha-reductase for the androgen substrate in the brain and undifferentiated gonads of *Malaclemys terrapin* turtles (Jeyasuria and Place, 1998). These reports documenting the thermal sensitivity of the developing brain, and a brain-gonadal axis of endocrine communication suggested that the neuroendocrine system could play an important role in TSD (Deeming and Ferguson, 1989b, 1991a; Jeyasuria and Place, 1998; Merchant-Larios, 1998; Eriksson *et al.*, 1999). However, the response of the gonads to temperature was found not to be mediated by the brain, implying that these are concordant, but not interdependent processes (Gutierrez-Ospina *et al.*, 1999). Interestingly, it has been found that yolk steroid hormones (which are maternally allocated) can affect sex ratios at least at the pivotal temperature in some TSD species (Bowden *et al.*, 2000; Elf, 2004).

Temperature-dependent sex determination

Johnston *et al.* (1995) reported the presence of SRY-type proteins (related to sex differentiation in GSD species) and their involvement in sexual differentiation in two species with TSD, the American alligator (*Alligator mississippiensis*) and the leopard gecko (*Eublepharis macularius*). Proteins encoded for by both SRY (human) and sry (mouse) have been postulated to control mammalian male development through the regulation of specific target genes, because SRY DNA-binding domain recognises the proximal upstream elements in the promoters of both P450 aromatase and the anti-Mullerian hormone (AMH), two compounds related to the sexual development of embryos (Haqq *et al.*, 1993; Johnston *et al.*, 1995). More recent studies in alligators found that AMH (also called MIS) expression precedes that of SOX9 in male embryos, while AMH expression was not detected in female embryos at any stage examined (Western *et al.*, 1999a, 1999b). SOX9 is member of a large family of SOX genes containing an SRY-like HMG box, and is related to testis differentiation in mammals (Morrish and Sinclair, 2002). SOX9 expresses differentially (higher at female than at male-producing temperatures) in the TSD lizard *E. macularius* (Valleley *et al.*, 2001). By contrast, SOX9 is upregulated in male embryonic gonads in turtles with TSD: *Trachemys scripta* (Spotila *et al.*, 1998) and *Lepidochelys olivacea* (Moreno-Mendoza *et al.*, 1999; Torres-Maldonado *et al.*, 2001) as well as in the American alligator (Western *et al.*, 1999b), although the onset of this differential expression occurs earlier in sea turtles than in alligator (Merchant-Larios, 2001). AMH has been cloned in *T. scripta* (Wibbels *et al.*, 1998).

WT1, the Wilms' tumour suppressor gene involved in human sex determination, shows no sexually-dimorphic expression in alligators (Western *et al.*, 2000). However, WT1 was cloned in *T. scripta* (Spotila *et al.*, 1998) and shown to produce two alternative splice isoforms whose ratio remains relatively constant, but with higher expression at male- than at female-producing temperatures during the stages examined.

The steroidogenic factor 1 (SF1), a gene required for the formation of mammalian primary steroidogenic organs (adrenal gland and gonad), expresses differentially during gonadal development in mammals and also at male and female producing temperatures in *T. scripta* (higher expression in males) (Fleming *et al.*, 1999; Crews *et al.*, 2001). SF1 expression at the pivotal temperature shows three distinct patterns in different individuals: male-like, female-like, or intermediate, and shifts from a male- to a female-producing temperature (or *vice versa*) during the TSP induces a corresponding change in gene expression with short-term overcompensation (Fleming and Crews, 2001).

Another factor involved in ovarian differentiation in mammals, DAX1 (Ramkissoon and Goodfellow, 1996) has recently been cloned in *A. mississippiensis* (Pieau *et*

N. Valenzuela

al., 1999; Western *et al.*, 2000; V.A. Lance, unpublished results) and in the turtle *Chrysemys picta* (Valenzuela, unpublished data). Western *et al.* (2000) found no differences in DAX1 expression between male and female alligator embryos, but this gene appears to express differentially at male and female temperatures in *C. picta* (Valenzuela, unpublished data). Additionally, a gene postulated as a candidate regulator of sexual development in vertebrates, DMRT1 (Morrish and Sinclair, 2002), shows higher expression in male *A. mississippiensis* (Smith *et al.*, 1999) and *T. scripta* (Kettlewell *et al.*, 2000) embryos, prior to sexual differentiation but after the onset of the thermo-sensitive period.

Nevertheless, finding these common factors between GSD and TSD species still does not tell us how TSD works in nature because no study has examined the effect that fluctuating temperature has on the expression of these genes. Additionally, most published reports have studied the differential expression/activity pattern of one (or a few) gene/enzyme(s) at a time under constant temperature after the onset of the thermosensitive period (but see Gabriel *et al.*, 2001). No data exist about the effect that fluctuating temperature has on the expression (individually or combined) of these regulators, and no simultaneous examination of TSD and GSD closely related taxa has been reported.

Evolution

Collectively, researchers have empirically explored the relationship between reptilian TSD and particular life-history parameters, environmental conditions, population genetic dynamics, and phylogeny of a limited set of taxa to evaluate the plasticity, significance, and evolutionary potential of this sex-determining mechanism (*e.g.* Joanen *et al.*, 1987; Gutzke and Crews, 1988; Swingland *et al.*, 1990; Ewert and Nelson, 1991; Janzen and Paukstis, 1991a, 1991b; Janzen, 1992, 1994, 1996; Burke, 1993; Bohn and Brooks, 1994a; Ewert *et al.*, 1994; Girondot *et al.*, 1994; Mrosovsky, 1994; Spotila *et al.*, 1994; Rhen and Lang, 1995, 1998; Roosenburg, 1996; O'Steen, 1998; St. Clair, 1998; Shine, 1999b; Valenzuela *et al.*, 2003) Space precludes any major review of these investigation but see the review by Valenzuela (2004). Though the biological significance of environmental sex determination seems clear for various taxa (Bull, 1983; Conover, 1984; Michaud *et al.*, 1999), the evolution of TSD in most reptiles and other vertebrates remains unexplained (Shine, 1999b; Valenzuela, 2004).

Theoretically, TSD can originate neutrally or as an adaptive response to selective pressures. The evolution of TSD (*i.e.* its divergence or loss) after its original establishment in a population or species could be neutral or adaptive, irrespective of the forces responsible for its origin. Existing evolutionary hypotheses (Table 9.2) relate to genetic or environmental effects (maternal and/or paternal) on gonadal

Temperature-dependent sex determination

sex and other phenotypic traits, which influence the survival or fecundity components of individual fitness or sex ratios. These hypotheses and other evolutionary considerations of the origin and maintenance of TSD in vertebrates in general and in reptiles in particular are reviewed in detail in Valenzuela (2004) and thus will not be described at length here.

Table 9.2. Theoretical models of TSD evolution. Case reports with positive, negative or equivocal support for each of these hypotheses are reviewed in Valenzuela (2004).

<i>Theoretical models</i>	<i>Source</i>
Adaptive TSD origin or maintenance	
Differential fitness	Charnov and Bull (1977)
Natal homing	Reinhold (1998)
Differential dispersal	Julliard (2000)
Differential mortality	Burger and Zappalorti (1988)
Sexual size dimorphism	Head <i>et al.</i> (1987); Webb <i>et al.</i> (1987)
Seasonal hatching time	Conover and Kynard (1981)
Phenotypic effects	Reviewed in Valenzuela (2004)
Nest-site choice by egg size	Roosenburg (1996)
Group structure adaptation	Bull and Charnov (1988)
Sib-avoidance	Ewert and Nelson (1991)
Group selection of sex ratio	Woodward and Murray (1993)
Cultural inheritance of natal homing	Freedberg and Wade (2001)
Adaptive TSD loss	
GSD individuals more fit	Bull (1981, 1983)
Sex ratio fluctuations	Bull (1980)
Intersexes	Bull (1981)
Allee effect	Berec <i>et al.</i> (2001)
Low/late dimorphism	Bull (1983)
Antagonistic pleiotropy	Moran (1992)
Imperfect phenotype-environment matching	Moran (1992)
Parental sex ratio control	Roosenburg (1996); Reinhold (1998)
Neutral TSD origin or maintenance	
Pre-existing Temperature sensitivity	Bull (1981)
Sex-ratio distorter	Morjan (2002)
TSD equivalent to GSD	Bull (1980); Mrosovsky (1980)
Phylogenetic inertia	Bull (1980)
Longevity	Bull and Bulmer (1989); Giron-dot and Pieau (1996)
Overlapping generations	Bull and Bulmer (1989); Giron-dot and Pieau (1996)

N. Valenzuela

From these hypotheses derive two sets of testable predictions (Valenzuela, 2004). Firstly, species could have TSD if a) sex ratio biases are advantageous, tightly correlated with temperature, and are unachievable under GSD; b) temperature affects fitness differentially for males and females either directly or through a factor highly correlated with temperature; c) fitness differentials between males and females are large enough, and conferred by environments frequently encountered in nature; or d) longevity, overlapping generations, and/or genetic variation for temperature sensitivity exists that buffers against sex ratio fluctuations rendering TSD neutral.

Alternatively, species should not have TSD if: a) sex ratios are drastically skewed at the generation time scale due to variation in environmental temperatures; b) sex ratios are highly biased due to the lack of enough thermal variation during offspring development; c) sex chromosomes are heteromorphic to such degree that they would produce lethal YY or WW at encountered temperatures; d) sex determination by temperature is genetically linked to a trait that is selected against; e) parents or offspring can control the patch that the offspring enter thus inducing frequency-dependent selection due to the environmental predictability and sex ratio control; f) low matching of phenotype to environment produces the wrong sex at a given temperature; g) temperature and the differential-fitness factor are decoupled such that there is a low correlation between the cue and the selective agent; or h) patches conferring differential fitness for males and females are uncommon, *i.e.* most environments encountered favour both sexes (from Valenzuela 2004).

Some concluding remarks are worth noting however. Conclusive evidence supporting adaptive explanations for TSD evolution in vertebrates is limited to very few species (*e.g.* the fish *Menidia menidia* and the lizard *Amphibolurus muricatus*). For most TSD reptiles, a neutral null hypothesis remains as the unfalsified explanation, and evidence of adaptive interpretations is for the most part consistent but inconclusive. Indeed, in many of the long-lived reptiles with overlapping generations, TSD is likely a trait maintained neutrally or quasi-neutrally, although long-term data is still required before a final conclusion can be reached. Importantly, heritability *sensu stricto*, a necessary component for adaptive evolution, is still undocumented at levels that allow selection to operate meaningfully. Lastly, more than one explanation for the evolution of TSD will likely be required, because TSD may have evolved by different means in different vertebrate groups, even within reptiles (Valenzuela, 2004).

Conservation

Many TSD reptiles are endangered most likely as the result of a combination of factors including overexploitation, habitat deterioration, and perhaps even TSD

Temperature-dependent sex determination

itself. Although the former two factors are the most threatening to the survival of TSD and other imperilled taxa, global warming could also be a meaningful hazard in the short- and long-term if climate changes much faster than the potential rate of evolution of TSD, nesting behaviour, or migration. Particularly, if habitat disturbance destroys suitable habitats or if fragmentation renders them inaccessible, the evolution of these traits to counterbalance climate change will be unachievable. The extinction risk of TSD taxa would be increased even further under small population sizes and short life-spans. Importantly, any management program for TSD taxa must take into account the thermal-dependence of the sex-determining system in such species when translocation of nests or artificial incubation are involved.

Conclusions

The incubation environment has effects at many levels in the biology of reptiles (see also Chapter 10). One remarkable effect is that of incubation temperature on sex, which is widespread in several reptilian groups. The exact extent of the prevalence of this sex-determining mechanism remains unknown because many reptiles have not been studied. The main difference among TSD systems relates to whether females are produced at only one extreme or at both extremes of the viable temperature range. However, subtle yet significant variation exists among individuals, populations and species in terms of the effect of particular temperatures on sex production.

The thermal mechanics of TSD under natural incubation conditions are not fully resolved as more needs to be learned about the effects of fluctuating temperature on development and sex differentiation in these taxa. Likewise, the effect that fluctuating temperature has on the expression of the molecular regulators of the sex differentiation cascade is still unstudied, as well as what makes this cascade thermosensitive in TSD taxa and not in GSD taxa.

Finally, because TSD taxa show no consistent genetic differences among the sexes, the expression of sex-related traits, Fisherian rules of sex ratio evolution, and recombination rules apply differently to TSD than to GSD, but the particulars of these differences are poorly known. All of these are open and active research areas, and the co-occurrence of TSD and GSD within Reptilia makes this an excellent clade for study. Integrative and comparative studies in this field will yield very interesting results with wide ecological and evolutionary implications.