Are Reptiles Predisposed to Temperature-Dependent Sex Determination?

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tive advantage to do so. Release of cryptic variation when canalization is challenged and fails at extremes may accelerate evolutionary transitions between GSD and TSD.

Abstract
Vertebrates show an astonishing array of sex determining mechanisms, including male and female heterogamety, multiple sex chromosome systems, environmental sex determination, parthenogenesis and hermaphroditism. Sex determination in mammals and birds is extraordinarily conservative compared to that of reptiles, amphibians and fish. In this paper, we explore possible explanations for the diversity of sex determining modes in reptiles, and in particular, address the prevalence of reptilian temperature-dependent sex determination (TSD) and its almost haphazard distribution across the reptile phylogeny. We suggest that reptiles are predisposed to evolving TSD from genotypic sex determination (GSD) by virtue of the uniquely variable thermal environment experienced by their embryos during the critical period in which sex is determined. Explicit mechanisms for canalization of sexual phenotype in the face of high thermal variation during development provide a context for thermolability in sex determination at extremes and the raw material for natural selection to move this thermolability into the developmental mainstream when there is a selec-

Sex determination has been a topic of speculation and rigorous inquiry since the time of Aristotle [Sturtevant and Lewis, 2001] and remains a hot topic today because of its intrinsic interest as a fundamental biological process and because greater understanding brings benefits for human health. For mammals and birds, sex is determined by a master sex gene suspected to act through dominance [Koopman et al., 1990; Sinclair et al., 1990] or dosage [Smith et al., 2007]. If we look beyond those 2 groups, vertebrates show an astonishing array of mechanisms that govern sexual phenotype. In many, environmental factors may interact with genotype [Conover and Kynard, 1981; Quinn et al., 2007; Radder et al., 2008] or act independently to determine sexual outcomes. Among reptiles with genotypic sex determination (GSD), male and female heterogamety (XX/XY and ZZ/ZW) is known in turtles, female heterogamety (ZZ/ZW, ZZ/ZZW, or ZZ/ZWW) is known in snakes and both are known in lizards (including XX/XXY) [Solari, 1993; Olmo, 2005]. Many species have GSD in the absence of any gross heteromorphy in sex chromosomes and many others have

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temperature-dependent sex determination (TSD) [Charnier, 1966; Bull, 1980; Ferguson and Joanen, 1982; Ewert et al., 1994; Cree et al., 1995; Harlow, 2004].

The systems of male heterogamety, female heterogamety and TSD have an almost haphazard distribution across the reptile phylogeny [Janzen and Krenz, 2004; Pokorna and Kratochvil, 2009]. At a high taxonomic level, birds have ZZ/ZW GSD, yet their sister class, the Crocodilians, universally have TSD. The sister families of turtles, Trionychidae versus Carettochelydidae and Chelidae versus Pelomedusidae, have GSD versus TSD systems, respectively. At lower taxonomic levels, the same pattern emerges. Within a single genus, *Amphibolurus norrisi* has GSD, yet its close relative *A. muricatus* has TSD [Harlow, 2004], and both systems are seen in populations of the Australian skink *Niveoscincus ocellatus* whose ranges differ in altitude [Wapstra et al., 2009]. As we learn more about the underlying mechanisms, and as our knowledge of the character states for sex determination becomes more refined, we can expect more cases of independent evolution of each of these states to come to light [Ezaz et al., 2009]. We can therefore anticipate a weaker, not stronger, phylogenetic signal to the pattern of systems of sex determination across reptile taxa.

A weak phylogenetic signal suggests that transitions between systems of sex determination in reptiles have been relatively frequent in their evolutionary history, and that, indeed, reversals between the 3 major systems of male and female heterogamety and TSD may well be relatively common. Furthermore, TSD is very common in reptiles, compared with the conservatism of the homeothermic birds and mammals which strictly control the thermal environment of the embryo by viviparity or behavioural means, and the poikilothermic frogs and fish whose eggs and larvae usually occupy relatively stable water-dependent thermal environments. The prevalence of TSD in reptiles under natural conditions, much more so than in other vertebrate groups [Chardard et al., 2004; Conover, 2004], raises the question of whether there is an underlying cause. *Are reptiles in some way predisposed to evolving TSD?*

In this paper, we view genotypic sex determination and the early stages of sex differentiation as labile at 3 levels. First, it can be genetically labile in the sense that variation among individuals in the sex genes and their regulatory signalling can redirect the developmental program to reverse sex from that expected by the presence or absence of a master sex determining gene. Second, it can be phenotypically labile or plastic in the sense that the sex of a reproductively functional individual can have been determined by the interaction of its genotype and the en-

vironment it experiences as an embryo. Third, it can be evolutionarily labile in the sense that transitions in the mechanisms of sex determination do not necessarily require major structural innovation in the genes governing sex determination.

We contend that thermostability of the regulatory systems of sex determination and differentiation in reptiles is achieved through well-developed buffering mechanisms, evolved explicitly in response to the highly variable thermal environment in which reptile embryos develop. These mechanisms render the regulatory system vulnerable to thermal influence when seriously challenged, such as when conditions move to encompass thermal ranges that are at the extreme of those that have been encountered by the species in its recent evolutionary history. We argue that release of cryptic genetic variability, selective advantages of thermolabile sex, and commonality in underlying processes of sex determination generally, have led to transitions between GSD and TSD in reptiles more frequently than in other groups that face less challenging thermal environments.

Sex Determination and Differentiation in Vertebrates

The terms sex determination and sex differentiation are often used in different ways and sometimes interchangeably [Valenzuela, 2008]. For the purposes of this article, sex differentiation is the development of the testes or ovaries from indifferent or undifferentiated gonads. This is not to be confused with sex determination, which is the process that directs differentiation to proceed down one or the other pathway, male or female [Hayes, 1998]. We regard sex to be determined once an embryo's fate is set under normal conditions of development; sex differentiation is the process that follows.

Under the classical view of GSD, sex in reptiles and other vertebrates is a phenotypic dichotomy driven by a more fundamental dichotomy in the genetic composition of males and females (X or Y chromosome complements in male heterogamety; Z or W in female heterogamety). Sex determination occurs at the time of conception. For example, sex is determined in most mammals by the presence or absence of a master sex determining gene, *SRY*, which resides only on the Y chromosome [Koopman et al., 1990; Sinclair et al., 1990]. Expression of this gene within the bipotential gonad, for a brief period early in embryogenesis, triggers a regulatory cascade that governs cellular differentiation and testicular gonadogene-

sis. Absence of a primary regulatory signal from *SRY* results in progression of the developmental program leading to an ovary. The expression of *SRY* in the primordial gonad gives effect to that defining event at conception – whether the zygote inherits or does not inherit a Y chromosome and the *SRY* gene. Thus the term sex determination is used in 2 ways, depending on context. In an organism with strict GSD, sex is determined at conception when its genotype is established, but we also refer to sex as being determined when that genotype initiates the regulatory process that directs sex differentiation down either a male or a female trajectory.

Birds have a female heterogametic system of sex determination (ZZ male, ZW female), but the genic mechanism has proven elusive [Clinton and Haines, 1999; Smith et al., 2007]. Like in mammals, sex determination is driven by a fundamental difference between male and female genotypes. It is thought that a Z master sex gene specific to the Z chromosome (probably *DMRT1*) [Smith et al., 2003] initiates male development when in double dose (ZZ) and female development when in single dose (ZW) [Smith et al., 2007], though the possibility of a W chromosome master sex gene has not yet been eliminated. A master sex determining gene is yet to be identified in an amphibian, but *DM-W*, a paralogue of *DMRT1* on the W chromosome, is involved in ovarian development in the frog *Xenopus laevis* and it has been proposed as a candidate sex determining gene in this species [Yoshimoto et al., 2008]. The only non-mammalian vertebrates for which the master sex determining gene has been discovered are 2 species of medaka fish (*Oryzias latipes* and *O. curvinotus)* in which a duplicated copy of *DMRT1* (referred to as *DMY* or *dmrt1bY*) was transposed, captured sex determination from an as yet unidentified master sex gene, and resulted in the establishment and differentiation of a neo-Y chromosome within the last 10 million years [Matsuda et al., 2002; Nanda et al., 2002].

The molecular basis of sex determination and differentiation in reptiles is poorly understood. In particular, a master sex gene has yet to be identified in any GSD reptile. There is not yet a model reptile GSD system to serve as a focus for coordinated attention in the way that the human, mouse and chicken have served this role. Research on genetic variants within single species that has led to so many remarkable insights in humans and mice has not occurred in reptiles, and the level of understanding of the reptilian system is not yet at a stage to allow experimental in vivo manipulation of the regulation of key genes, as has been achieved for mammals and birds [Smith et al., 2007; Wilhelm et al., 2007].
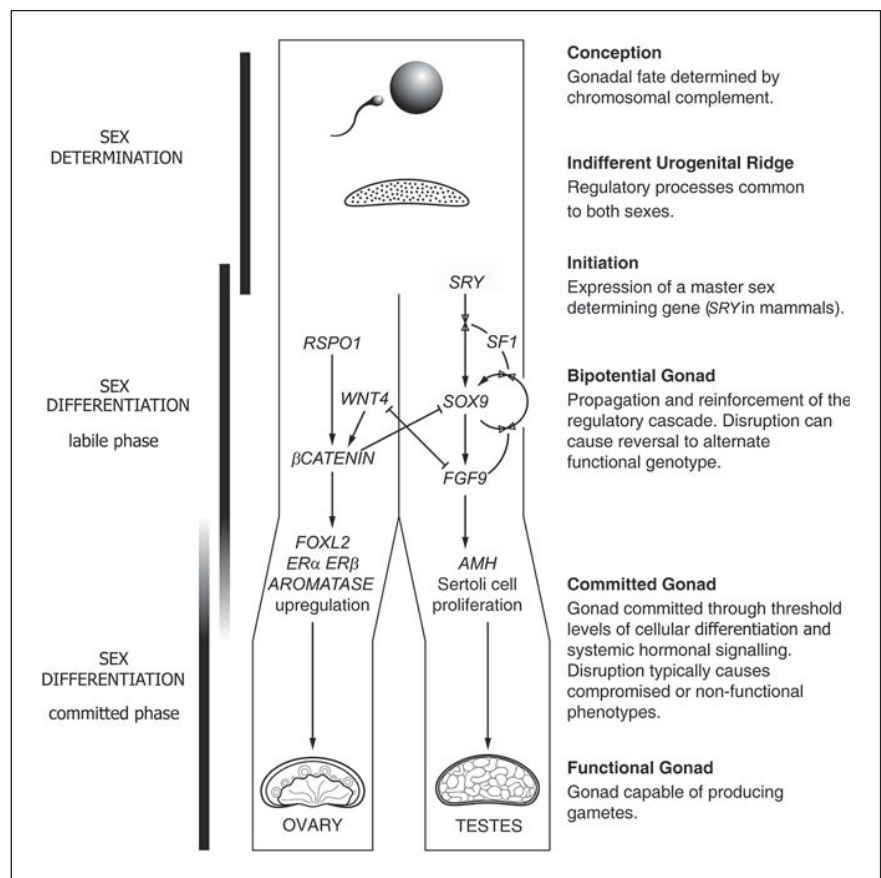
The Labile Phase of Sex Differentiation

There is a period, early in the process of sexual differentiation, and arguably including the expression of any master sex gene, when the sexual fate of the embryo is vulnerable to influences of the broader intracellular and extracellular regulatory environment. We refer to this as the *labile phase* of sex differentiation. During this phase, sex differentiation is genetically labile in the sense that variation among individuals in the genes governing differentiation can result in sex reversal to functional sexual phenotypes. Under strict GSD as in mammals, where extrinsic environmental influences are usually negligible, sex is determined by a master sex gene in the context of the individual's broader genotype (including any autosomal or sex-linked modifiers) which is also set at conception. Thus, although labile among individuals of GSD species, sex differentiation is not necessarily labile within a single individual undergoing normal development.

We know from studies of mammals, and to a lesser extent birds and fish, that this early stage of sex differentiation is characterized by a complex network of regulatory genes and their interactions, including forward initiation [Sekido and Lovell-Badge, 2008] and feedback loops [Sekido et al., 2004; Kim et al., 2006; Sekido and Lovell-Badge, 2008] (fig. 1). Such feedback loops are alone sufficient to commit development to 1 of 2 or more discrete developmental trajectories [Becskei et al., 2001; Ferrell, 2002; Chatterjee et al., 2008]. However, the early phase of sexual differentiation in both sexes is also characterized by cross-program suppression, including antagonistic interactions between pairs of genes involved in the competing male and female developmental programs [e.g. *FGF9* and *WNT4*; Kim et al., 2006], that reinforce the developmental trajectory, male or female (fig. 1).

Once distinct cellular structures begin to emerge, intercellular and hormonal signalling become more prominent and the 'decision' on sexual fate becomes irreversible in the sense that genetic variation among individuals is likely to generate gonadal structures and phenotypes that are aberrant and non-functional. Sex differentiation has entered the *committed phase* coinciding with the structural phase of gonadogenesis. This *committed phase* of sex differentiation is likely to show less variation in the genes that govern it, or greater canalization in their overall influence on sexual phenotype, than does the *labile phase*, sustained by natural selection acting against variation that leads to non-functional or fitness-impaired sexual phenotypes.

Fig. 1. A schematic diagram of sex determination and sex differentiation in a GSD species. Sex is determined at conception by the chromosomal complement of the zygote. An indifferent gonadal ridge differentiates into a bipotential gonad when the master sex determining gene (*SRY* in mammals, as shown) initiates the regulatory cascade that directs and maintains sex differentiation, male or female. Forward initiation, positive feedback, cross-program suppression including reciprocal antagonism, are illustrated using examples known from mammalian systems. Perturbation of the labile phase of sex differentiation by genetic variation in the sex genes and the genetic environment in which they are expressed, or by environmental influences, can cause a system switch between the male and female developmental programs leading to functional sexual phenotypes. Perturbation during the committed phase, thought to occur once cellular differentiation is underway, typically leads to compromised or non-functional sexual phenotypes. In thermolabile systems that lack a master sex determining gene, sex determination is less well defined, and occurs when the regulatory system during the labile phase of sex differentiation is irreversibly displaced in favour of one pathway or the other.

Thus, in reptiles with GSD, as in mammals and other vertebrates with GSD, we can expect a *labile phase* of sex differentiation beginning with expression of a master sex determining gene and propagation of the primary regulatory signal to initiate the chosen developmental trajectory. Genetic variation, or indeed extrinsic environmental influences, presumably alter levels of expression of key sex genes and so influence the strength of the sex-determining regulatory signal, the efficacy by which it is propagated within the cell or between cells, or the efficacy of its reception. This genetic variation will thus affect gonadal fate in particular individuals and provide the raw material for natural selection to drive transitions between sex determining modes. Selection will be tolerant of such genetic variation or environmental influences during the labile phase because displacement of the regulatory system during the labile phase will typically lead to functional sexual phenotypes, *albeit* sometimes reversed with respect to chromosomal sex. The possibility of compromise of the functional phenotypes because of such rever-sal is least early in the execution of the developmental program and increases as the program progresses through to the point where the system can be regarded as committed to one or the other sexual phenotype. As such, variation in genes engaged early in sex differentiation is most likely to be tolerated by natural selection (less likely to be deleterious), and so most likely to be involved in transitions between systems of sex determination [see also Wilkins, 1995].

Plasticity of Sexual Phenotype

Environmental sex determination is an example of plasticity in a discrete phenotypic trait. Species with strict TSD differ fundamentally from those with GSD in that phenotypic males and females have no consistent difference in genotype. No master sex gene unilaterally determines gonadal sex through dosage or dominance. There are no heteromorphic chromosomes differentially co-oc-

curring with phenotypic sex. While in strict GSD, individuals undergoing normal development are not equipotent, having had sex determined at conception, in TSD the point when sex is determined is not precise. In TSD, much of the process that is homologous to the *labile phase* of sex differentiation in GSD species can precede sex determination, and individuals are considered equipotent for a protracted thermosensitive period that typically spans the middle third of development [Yntema, 1979; Bull and Vogt, 1981; Mrosovsky and Pieau, 1991; Young et al., 2004].

It is tempting to postulate that sex determination in TSD reptiles is governed by a master sex determining gene with expression that is temperature-sensitive and that operates as a switch. Such a master TSD sex gene could be ancestral, or could be one that has captured sex determination from an ancestral master GSD sex gene which is subsequently lost from the population (dominance system) or rendered of constant dose in both sexes (dosage system) through YY or WW lethality [Bull, 1985; Sarre et al., 2004]. A more likely scenario in our view, given the thermosensitivity of almost all simple chemical and enzymatic processes, is that there is a system-wide response of the gene regulatory system to temperature during the labile phase of sex differentiation in TSD species. Relaxation of the control of a master sex gene and associated processes leading to canalization of the sex determining regulatory signal it initiates, would allow this inherent thermolability to manifest. Under this 'parliamentary system' [Crews and Bull, 2009] of sex determination, it is the regulatory communication among the network of sex genes that is displaced by temperature, and ultimately it is the integrated signal at the point of transition of the system from the *labile phase* to the *committed phase* of sex differentiation that determines sex. That signal will depend in part on conditions that apply at the time of such transition, on how far the regulatory system has been displaced by the often variable temperatures experienced by the system leading up to that point, and the rate at which the influence of such thermal history on the regulatory system dissipates. Sex in TSD species is determined when the regulatory system is displaced unrecoverably in the direction of one developmental program or the other, male or female, and its timing will depend on thermal history in the context of the individual's genetic background. This view of sex determination in TSD species offers a potential explanation for the response of TSD systems to temperature pulses during the thermosensitive period, which manifests as a form of cumulative influence of temperature [Delmas et al., 2008; Girondot et al., this issue].

Canalization of Sexual Phenotype

We have argued that during the early labile phase of sex determination and differentiation, the regulatory program is vulnerable to perturbations arising either intrinsically through interindividual variation in the regulatory sex genes or the genetic background in which they function, or arising extrinsically through intraindividual influences of the thermal environment. The regulatory program of strict GSD species, where sex remains concordant with chromosomal sex established at the time of conception, must be resistant to these perturbations, that is, the male and female regulatory programs must each be strongly canalized [sensu Waddington, 1942].

Both empirical and computational evidence suggests that complex evolved networks are extremely robust to perturbation [Siegal and Bergman, 2002], and that more densely connected networks are often associated with increased canalization and developmental stability [but see Leclerc, 2008]. Contributing to that stability is the possible involvement of molecular chaperones, such as the ubiquitous heat shock proteins (HSP). For example, HSP70 interacts with a highly conserved region of the SOX9 protein [Marshall and Harley, 2001], suggesting it maintains the functional conformation of the complex of proteins vital to the role of SOX9, stabilizing its regulation of sex differentiation and other developmental processes in which it is involved [Marshall and Harley, 2001]. Some conserved microRNAs may also be involved buffering developmental programs against thermal variation and imparting robustness to developmental regulatory networks [Hornstein and Shomron, 2006; Li et al., 2009]. Other mechanisms that stabilize the development of discrete structures in the face of genetic or environmental variation have been proposed, including redundancy in gene function and overproduction of regulatory elements that would otherwise become rate limiting [Whittle, 1998; Kitano, 2004; Flatt, 2005].

Reptiles, perhaps more than other vertebrates, are particularly challenged by the thermal environment in which their embryos develop. Mammalian embryos are maintained under conditions of homeothermy through residence within the womb or pouch when sex is determined. Although not as strictly controlled, temperatures of the bird embryo are maintained within relatively narrow limits by the parent using body heat [White and Kinney, 1974], heat generated from rotting vegetable material [megapodes, Seymour and Bradford, 1992], or heat from other sources [de Marchi et al., 2008]. In contrast, the reptile embryo needs to accommodate a wide range of
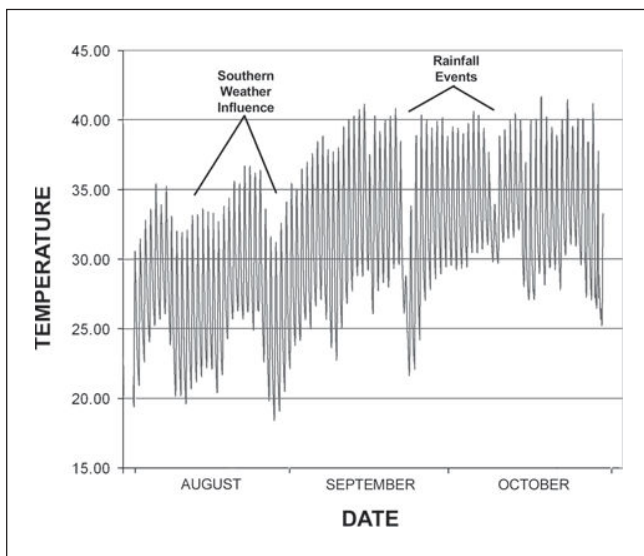
Fig. 2. A temperature trace for the complete incubation of a nest of the pig-nosed turtle *Carettochelys insculpta* (TSD) in the 1998 breeding season (Trace N9830T). Note the seasonal trend in temperature, the pronounced daily cycle in temperature and the effect of weather all of which make for a complex and highly variable thermal regime during embryogenesis. The embryo and the regulatory system governing normal development must contend with temperatures ranging from below 20°C to above 40°C.

temperatures during the critical stages of sex determination. The thermal environment varies among nests depending upon where and when they are laid. Temperatures they experience vary from year to year and as the season progresses within a single year. Even diel fluctuations in temperature can be substantial [routinely ranging up to 15°C in a single day in temperate latitudes; Pieau, 1982]. In the tropical turtle *Carettochelys insculpta,* embryos completing normal development can anticipate an average temperature of around 32°C, but must contend with the possibility that they will routinely experience temperatures as low as 24°C and as high as 45°C [Georges and Doody, unpublished data]. For example, in 1998, mean temperatures varied among nests by up to 4.6°C (n = 58), the average daily range in temperature within nests was 5.7°C (2.4 to 11.1°C), and eggs within single nests experienced seasonal shifts in temperature of up to 8.8°C (fig. 2). Additional sources of thermal variance derive from interannual and geographic variation in weather conditions. This routinely experienced thermal variation must present a formidable environmental challenge to the regulatory processes governing development.

Even the embryos of viviparous reptiles must contend with a wide range of temperatures during development, because gravid females can maintain high temperatures within relatively narrow limits only while active. The regulatory system that directs sex determination in GSD reptiles and the process of gonadogenesis that follows needs to be robust to temperature variation if a regulatory signal giving effect to sex determination at conception is to propagate consistently and effectively. Reptiles with GSD therefore require well-developed mechanisms for achieving canalization of the regulatory signal directing sex determination and differentiation to achieve developmental stability, arguably more so than those required by the homeothermic mammals and birds or other groups whose embryos develop under relatively stable thermal conditions.

Whatever the mechanism in place for buffering sex determination and early differentiation against high thermal variation among nests and thermal variability within reptile nests, one can expect it to be fully functional only across the range of thermal conditions experienced during the recent evolutionary history of the species. The organism must also balance achieving thermostability of the genetically-directed sex regulatory system against the many other functions of the relevant genes and their expression in the developing embryo [Purugganan and Gibson, 2003; Williams et al., 2003]. When challenged by novel environmental conditions, the mechanisms affording thermal stability can be expected to fail under some circumstances, because temperatures range beyond those to which the buffering system is adapted.

Evidence for the failure of mechanisms that afford thermal stability can be found in GSD amphibians where extremes of temperature have long been known to affect sex ratios [Dournon and Houillon, 1984; Hayes, 1998; Nakamura, 2009]. The temperatures applied in such experiments are outside those normally experienced in the wild, and the sex reversal is usually interpreted as an artefact of abnormally high temperatures [Schmid and Steinlein, 2001]. In our lab, we pushed the thermal environment of eggs of the dragon lizard *Pogona vitticeps* to extremes [Quinn et al., 2007]. This species has a ZZ/ZW system of genotypic sex determination with sex microchromosomes [Ezaz et al., 2005]. High incubation temperatures caused reversal of the ZZ genotype to yield phenotypic females (fig. 3) [Quinn et al., 2007, 2009a] suggesting that buffering of the male-developmental program from temperature variation had failed, and that a default female program had prevailed. Sex reversal in the wild has not yet been demonstrated, but seems likely as tem-

peratures as low as 34°C are sufficient to cause some reversals. A similar case has been documented in the Australian skink *Bassiana duperreyi*, which has an XX/XY system of sex determination, yet produces a male-biased sex ratio at lower temperatures [Shine et al., 2002]. Sex reversal was subsequently confirmed using sex linked markers [Radder et al., 2008; Quinn et al., 2009b]. In this case, the sex-reversing temperatures are within the range of temperatures experienced in the wild [Radder et al., 2008]. In these species, sex determination is concordant with genotype across a wide thermal range (GSD), but sex is reversed to functional phenotypes at one extreme temperature (fig. 3). This is strongly suggestive of effective canalization of the sexual program at intermediate but highly variable temperatures during the labile phase of embryonic development, canalization that is compromised and ultimately fails as temperature is shifted to an extreme.

Transitions from GSD to TSD

Evolution of well-developed mechanisms for canalizing sexual phenotype in the face of high thermal variability during development has 2 consequences. First, when such a system is taxed, inherent thermolability of the regulatory systems during the early labile phase of sex determination and differentiation will be expressed, and so become available for evolution under natural selection or drift. The specific advantages of TSD over GSD that would drive such transitions have been a topic of active debate for several decades [Charnov and Bull, 1977; Janzen, 1996; Girondot and Pieau, 1999; Shine, 1999; Valenzuela, 2004] and were recently examined explicitly in experimental manipulations [Janzen, 1995; Warner and Shine, 2005, 2008].

Second, challenging the mechanisms of canalization can potentially release cryptic variation in the genes governing regulatory signalling, variation that has accumulated but with its expression suppressed by canalization of the phenotypic outcomes. Variation in the activity of the gene *HSP90* has major and wide-ranging effects on phenotype of some insects and plants [Rutherford and Lindquist, 1998; Queitsch et al., 2002]. These novel phenotypic variants have been interpreted as arising from a pre-existing genetic polymorphism that is normally hidden [Sangster et al., 2004]. For example, larval developmental rate in *Drosophila melanogaster* increases with temperature, as in many ectotherms. This parameter is unresponsive to artificial selection pressure at most tem-
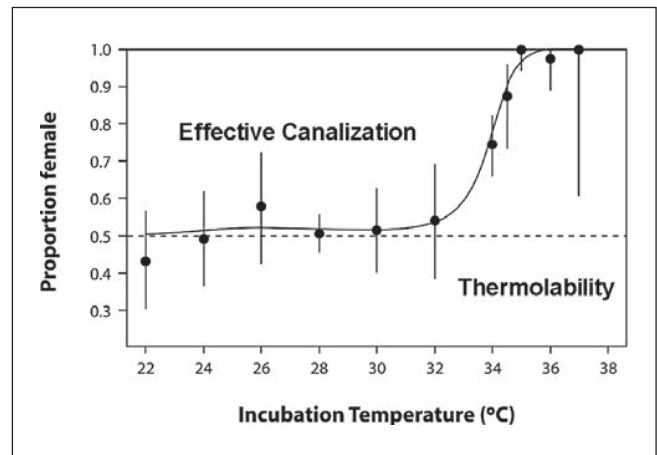

Fig. 3. Response of sexual phenotype in the Australian dragon lizard *Pogona vitticeps* [Quinn et al., 2007]. Sexual phenotype is concordant with ZZ or ZW genotype across a wide range of temperatures (22–32°C). This thermostability indicates strong canalization of the regulatory processes governing sex determination and differentiation. The mechanisms responsible for this thermostability fail at the higher extremes, leading to sex reversal.

peratures, but when larvae are raised under conditions of heat-stress (32°C), selection yields heritable increases in development rate that persists at both high and normal temperatures [Neyfakh and Hartl, 1993]. This greater evolutionary response under heat stress presumably arises through selection acting on novel genetic variation released by overstretching the molecular chaperones and associated buffering capacity of the developmental networks [Sangster et al., 2004]. Embryonic developmental rate and its relationship to temperature is a key ingredient in sex determination in shallow-nesting reptile species [Georges, 1989; Georges et al., 1994], and processes influencing its evolvability would have implications for evolutionary responses to environmental change. Thus, the evolution of TSD from GSD through intermediate forms where developmental buffering is taxed at extremes may be facilitated by coincidental release of cryptic genetic variation. This could include synchronous release of novel multiple polymorphisms [Sangster et al., 2004], a few of which may be advantageous and influential in sex determination. There could also be manifestation of ancestral thermolabile sex determining genes or pathways retained but not expressed under a more recently evolved GSD system, the existence of which has recently been suggested by Valenzuela [2007].

In conclusion, we argue that reptiles are predisposed to the evolution of TSD from GSD by virtue of the high variability in the thermal environment experienced by the developing embryo within and across nests, compared with that experienced by mammalian, avian, amphibian and fish embryos and larvae, and the possession of well advanced but nevertheless limited mechanisms for canalization of sexual phenotype in the face of such high thermal variability. When these buffering mechanisms are taxed, expression of inherent thermolability in the regulatory networks governing sex determination and early differentiation provides the foundation for evolving from GSD, to GSD with sex reversal at extremes, to TSD. These transitions may be assisted by coincident release of cryptic variation in the genes governing sex determination that has accumulated in the context of highly canalized sexual phenotypes. These processes may in part explain the relatively high frequency of TSD across reptile lineages and its apparent multiple independent origins. Release of cryptic variation when canalization is challenged by environmental stress, such as under climate change, may accelerate evolutionary transitions between GSD and TSD.

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