

MINI REVIEW

Exploring the evolution of environmental sex determination, especially in reptiles

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Abstract

Environmental sex determination has been documented in a variety of organisms for many decades and the adaptive significance of this unusual sex-determining mechanism has been clarified empirically in most cases. In contrast, temperature-dependent sex determination (TSD) in amniote vertebrates, first noted 40 years ago in a lizard, has defied a general satisfactory evolutionary explanation despite considerable research effort. After briefly reviewing relevant theory and prior empirical work, we draw attention to recent comparative analyses that illuminate the evolutionary history of TSD in amniote vertebrates and point to clear avenues for future research on this challenging topic. To that end, we then highlight the latest empirical findings in lizards and turtles, as well as promising experimental results from a model organism, that portend an exciting future of progress in finally elucidating the evolutionary cause(s) and significance of TSD.

Introduction

Sex-determining mechanisms (SDMs) can be categorized according to the primary causal agent of sex determination. Under environmental sex determination (ESD), sex is established by a nongenetic cue (e.g. nutrient availability) experienced within a discrete period after conception (Bull, 1983). In contrast, genotypic sex determination (GSD) occurs when sex is established by genetic factors (e.g. sex chromosomes) at conception. Understanding and distinguishing between ESD and GSD is important because the evolutionary dynamics of genes and primary sex ratio, for example, should differ fundamentally between these two categories of SDMs (Valenzuela *et al.*, 2003).

The great diversity of SDMs has thus long puzzled evolutionary biologists. It is easy to envision how 1 : 1 primary sex ratios and GSD are selectively linked. Because each sex must provide half the genes to

subsequent generations, frequency-dependent selection on the primary sex ratio is strong (Fisher, 1930) and so meiotic separation of sex chromosomes provides a painless solution to the problem. It is perhaps surprising then to learn that ESD (and, potentially, adversely biased sex ratio) is phylogenetically widespread, occurring in such diverse taxa as plants, nematodes, echiurid worms, amphipods, fish and amniote vertebrates (Korpelainen, 1990,1998). Despite the possible Fisherian fitness cost of biased sex ratio under ESD, the selective pressures favouring ESD over GSD in most of these taxa are understood (Bull, 1983). For instance, photoperiod induces production of males earlier, and females later, in the season in the amphipod (*Gammarus duebeni*). Because male fitness improves more than female fitness with increased size, ESD is adaptive in this system by permitting males to experience a longer growing season than females (McCabe & Dunn, 1997). The adaptive significance of ESD is not so clear for amniote vertebrates, however, the clade in which this SDM is perhaps most common.

Most amniote vertebrates have GSD, but a small highly diverse set of taxa in this clade possesses ESD. In these latter animals, ESD occurs as temperature-dependent sex

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determination (TSD), wherein sex of individuals is permanently determined by thermal conditions experienced approximately during the middle one-third of embryonic development (Janzen & Paukstis, 1991a; Valenzuela & Lance, 2004). Mammals and birds only have GSD (Bull, 1983), whereas crocodylians and sphenodontians exclusively have TSD. In contrast, squamates (lizards and snakes) and turtles exhibit both GSD and TSD, although the latter SDM is much more common in turtles than in squamates (Fig. 1; Janzen & Krenz, 2004). Despite the frequency of TSD in amniote vertebrates and substantial research efforts, clarifying the evolutionary dynamics of SDMs in this clade remains a considerable challenge. Such research has practical implications as well, since a large fraction of imperiled amniote taxa have TSD (e.g. sea turtles, crocodylians, sphenodontians) and these groups frequently are threatened by rapid climate and habitat change that could affect nest thermal environments and, hence, offspring sex ratios (e.g. Janzen, 1994).

In this review, we evaluate the evolution of TSD in amniote vertebrates, emphasizing theory and research concerning this enigmatic SDM. We begin with a focused assessment of the relevant theory along with a brief overview of the (lack of) general supporting evidence in this clade for the adaptive significance of TSD relative to GSD. We then draw attention to recent phylogenetic comparative analyses of SDM evolution in amniote vertebrates. These findings provide both a new reasonable explanation for the generally unsatisfying results of prior empirical work on the adaptive significance of TSD in this clade and a fresh prescription to direct future research endeavours on this important topic. Finally, we describe intriguing new empirical results that validate predictions and research directions identified by the comparative analyses and report exciting preliminary findings regarding the utility of experimental evolution approaches with an engineered model organism for exploring the evolutionary dynamics of TSD.

Adaptive significance of temperature-dependent sex determination

Despite elegant theoretical work and several decades of empirical examination in this context, we are not obviously closer to a clear, general solution to the adaptive significance of TSD in amniote vertebrates. Rather than attempt an exhaustive account of this extensive body of work (reviewed in Shine, 1999), we instead focus here mainly on what we perceive to be the most fruitful avenues for making progress in solving this enduring enigma.

At least four general explanations for the adaptive significance or persistence of TSD in amniote vertebrates have been identified (modified from Ewert & Nelson, 1991):

1. Phylogenetic inertia – TSD is the ancestral condition in this clade and is simply maintained in extant lineages because it is currently adaptively neutral or nearly so.
2. Group adaptation – TSD promotes adaptive control of sex ratio to promote group fitness.
3. Inbreeding avoidance – TSD minimizes inbreeding by producing single-sex clutches.
4. Differential fitness – TSD provides sex-specific fitness benefits such that some developmental temperatures are better for one sex.

Little if any evidence exists for the first three explanations (Shine, 1999), although admittedly minimal effort has been expended to address them empirically (Burke, 1993). Instead, attempts to explain the adaptive significance and maintenance of TSD have largely revolved around aspects of differential fitness, whose primary theoretical framework was provided by Charnov & Bull (1977). Empirical work employing the Charnov–Bull model has focused mainly on the differential fitness condition of their verbal framework; it is the crux of the model and is often misunderstood. This condition can perhaps best be grasped graphically (Fig. 2). In words, however, this key aspect of the model requires that the ratio of male to female fitness must vary with temperature for TSD to evolve adaptively.

The Charnov–Bull model, which is a specific case of the more general model for when fitness varies with environmental conditions (e.g. West & Sheldon, 2002), has been profitably applied to explain the adaptive significance of ESD in a wide variety of taxa (Bull, 1983), including a fish with TSD (Conover, 1984). We believe the evidence is not so clear-cut in reptiles, however. Indeed, our view of the evidence is that a number of serious, thoughtful biologists have unsuccessfully employed this theoretical framework to evaluate the adaptive significance of TSD in various amniote taxa over the past 30 years: either a positive pattern detected in one species is not observed in another (e.g. Roosenburg, 1996; Morjan & Janzen, 2003), putative general explanations have substantial empirical exceptions (e.g. Ewert & Nelson, 1991; Janzen & Paukstis, 1991b), or a particular approach with positive findings has been attempted in only one species (e.g. Janzen, 1995).

To illustrate the state of the field, let us briefly consider what some researchers contend is the strongest evidence in favour of (or at least consistent with) the Charnov–Bull model in amniotes with TSD. Under this view, the sex that benefits most from enhanced growth should be produced at the incubation temperature that most improves post-hatching growth. We believe, however, that a more circumspect assessment of the presumptive evidence is in order. For every report that the sex under stronger selection is produced at incubation temperatures yielding maximal post-hatching growth (e.g. in the map turtle, *Graptemys ouachitensis*, Freedberg *et al.*, 2001), there is a study that describes a contrasting result under the same selection scenario (e.g. in the box turtle,

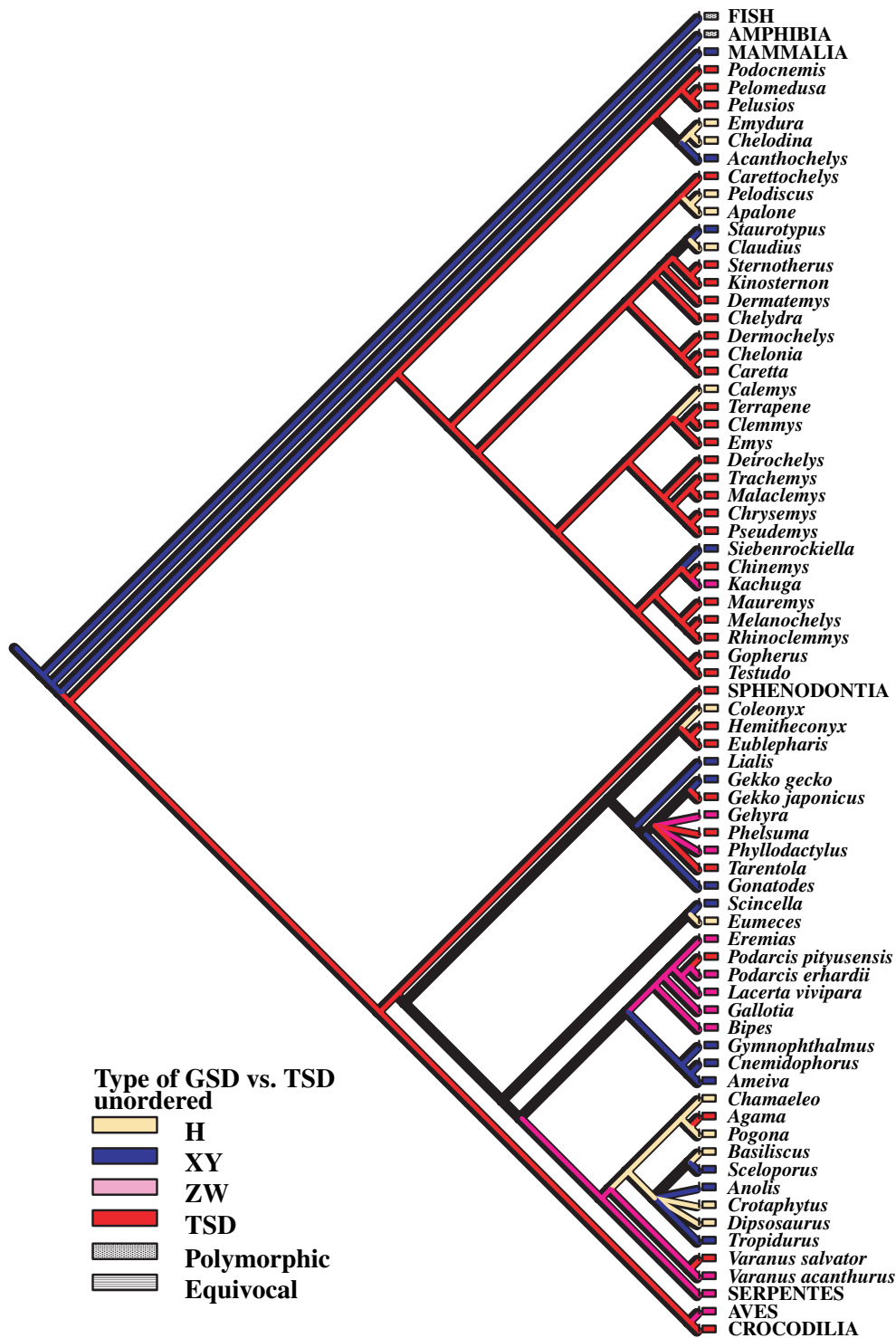


Fig. 1 Parsimony analysis of SDM evolution on a tetrapod vertebrate phylogeny. The basis of the construction of the phylogeny, information on SDMs and the methodology underlying the comparative analysis are reported in Janzen & Krenz (2004). H = homomorphic sex chromosomes, XY = male heterogamety and ZW = female heterogamety. Note the ancient origin of TSD (red = TSD) in amniotes as well as presumably more recent origins of TSD in squamates (e.g. *Agama*).

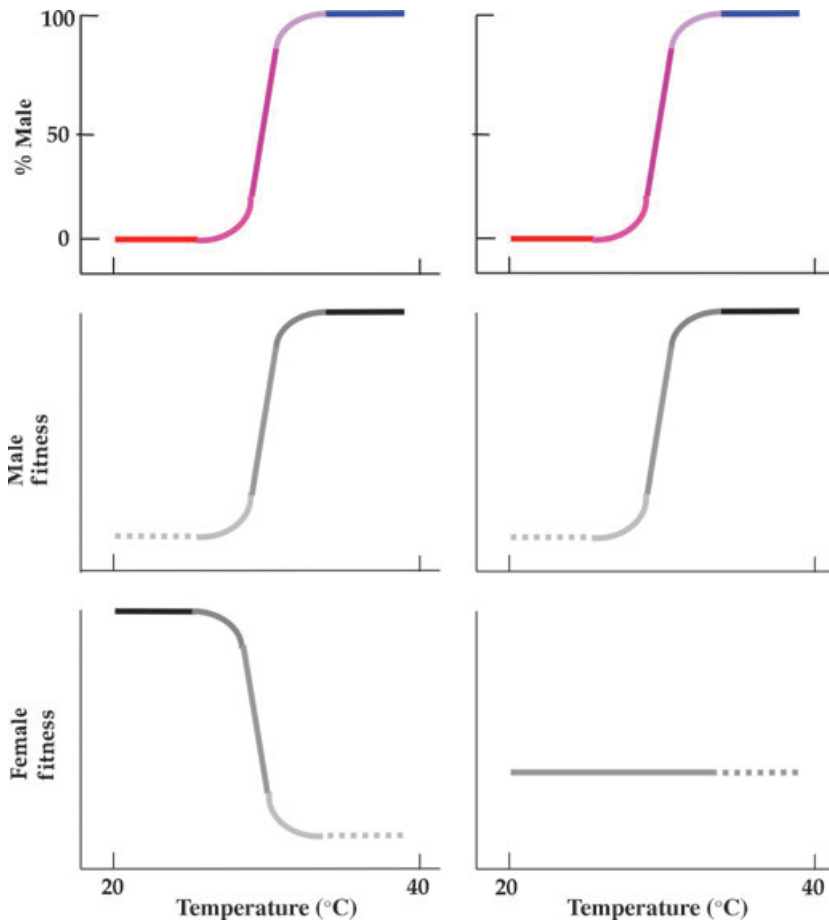


Fig. 2 Visualizations of the differential fitness condition of the Charnov–Bull model (Charnov & Bull, 1977). The top panels illustrate a pattern of TSD whereby offspring sex ratios vary continuously (from red = 100% female to blue = 100% male) as a function of different constant incubation temperatures. Based on this pattern of TSD, male fitness might be expected to be greater than female fitness under conditions that produce more male offspring (center panels); the converse might be true for female fitness (bottom panel on left) or female fitness might be independent of temperature (bottom panel on right) according to the Charnov–Bull model. Dashed lines indicate that individuals of the sex illustrated are not produced in that temperature range and thus fitness cannot be evaluated without experimental manipulations.

Terrapene Carolina, St. Clair, 1998). Conflicting findings are even obtained for the same species. For example, faster post-hatching growth has been detected in snapping turtles (*Chelydra serpentina*) from eggs incubated at more male-producing temperatures (e.g. Rhen & Lang, 1995). This result is thought to be consistent with the Charnov–Bull model because *C. serpentina* exhibits a pattern of large-male sexual dimorphism in adulthood. In contrast, using a more rigorous design in which turtles were reared individually rather than in artificial groups, Steyermark & Spotila (2001) found no relationship between post-hatching growth and incubation temperature in *C. serpentina*. While we do not advocate abandoning this line of research, the inconsistency of results between studies fuels our contention that alternative approaches deserve increasingly serious consideration.

These results for amniotes probably do not reflect any inadequacy of the Charnov–Bull model, but rather may derive from the evolutionary history of SDMs in this clade. Indeed, recent phylogenetic comparative analyses suggest a reasonable explanation for the empirical tumult in this field (Janzen & Krenz, 2004). These parsimony-

based analyses imply a single ancient origin for TSD in most amniote vertebrates around 300 million years or so ago on Pangaea, with several more recent independent origins of TSD in squamates (Janzen & Krenz, 2004; sensu Fig. 1). Consequently, the adaptive significance of TSD in all but the most recent origins of this SDM may have been obscured by the passage of deep time, with TSD potentially being maintained in many amniote clades simply because it ‘works’ (i.e. has no overall fitness costs along the lines of the phylogenetic inertia explanation). Consistent with this view, all efforts to explore the adaptive significance of TSD in amniote vertebrates have, with two recent exceptions (see below), focused on turtles, crocodylians, or gekkotan lizards (Valenzuela & Lance, 2004), clades all identified in the phylogenetically based comparative analyses with the original evolution of TSD in amniote vertebrates (Janzen & Krenz, 2004).

So where do we go from here?

The recent phylogenetic comparative analyses of SDM evolution in amniote vertebrates at the minimum

provide us with the hopeful, rigorous explanatory framework described in the previous section. But this comparative approach also reveals promising avenues for future research that should accelerate progress on the important issue of the possible adaptive evolutionary dynamics of TSD in amniotes. In particular, these comparative analyses suggest that (1) empirical efforts to evaluate the Charnov–Bull model in amniotes probably should be directed towards taxa in which TSD has evolved more recently and (2) any explanation for the adaptive significance or maintenance of TSD in turtles, crocodylians, etc. could reside outside the general framework of the Charnov–Bull model. We address these prospects in turn below.

Recent origins of temperature-dependent sex determination

As described above, phylogenetic comparative analyses employing a parsimony criterion suggest that TSD has evolved multiple independent times in squamates (Janzen & Krenz, 2004). Consequently, this comparative approach can be used to identify crucial taxa to target for further empirical work. To be specific, the more recent origins of TSD in amniotes appear to reside at least in agamid, lacertid and varanid lizards, with the most rigorous indisputable research documenting SDMs occurring for agamids (Harlow, 2004). This latter result is particularly fortunate, as two recent efforts to evaluate the adaptive significance of TSD in the context of the Charnov–Bull model have involved an agamid lizard.

Agamid lizards exhibit TSD wherein cool incubation temperatures produce females and warm incubation temperatures produce males (Viets *et al.*, 1994), although in at least some species the very highest incubation temperatures produce females again (Harlow, 2004). Consequently, under the Charnov–Bull model, one possible prediction in the latter instances is that females should have higher individual fitness when deriving from the coolest and warmest (rather than intermediate) incubation conditions and vice versa for males, if TSD is adaptive in these lizards. Harlow & Taylor (2000) began to address this very issue in the jacky dragon (*Amphibolurus muricatus*), a short-lived, early-maturing species (at least in comparison to turtles and crocodylians; Janzen & Paukstis, 1991a). Of particular interest, they noted that nests laid earlier in the season tended to produce male-biased sex ratios. The authors went on to suggest that this pattern was adaptive because males of this relatively short-lived species would benefit more than females from the opportunity for extra growth prior to hibernation, which should accelerate the subsequent onset of reproductive maturity in those males.

While a valuable contribution to the field, the adaptive interpretations of Harlow & Taylor (2000) study nonetheless rest on a sample size of just three nests. Sure enough, Warner & Shine (2005) conducted an extensive

manipulative experiment on the same species that reached contrasting conclusions! In this more recent experiment, eggs of *A. muricatus* were incubated at three temperatures, with the two most extreme developmental environments naturally producing only female offspring and the intermediate temperature producing about equal numbers of male and female offspring. However, the authors cleverly treated another subset of eggs in each incubation environment with fadrozole, an aromatase inhibitor, in order to produce at least some male offspring under all conditions. In this way, they could more clearly address the sex-specific differential fitness predictions of the Charnov–Bull model and evaluate alternative mechanisms that could support this model. After eliminating three of the four possible mechanisms posed in the context of their study, the authors identified hatching time as the likely driver of differential fitness between the sexes in this species. In contrast to the results of the earlier study on *A. muricatus*, Warner & Shine (2005) found that early hatching (i.e. naturally female) offspring in semi-natural enclosures grew substantially more prior to winter than later-hatching (i.e. primarily male) offspring. These females, in fact, essentially reached the minimum size for maturity by the time hibernation began, implying that they would be able to reproduce in their second year, nearly a year earlier than their conspecifics from cooler, later-hatching nests. The authors interpreted this pattern as adaptive because any early-maturing males would still be unlikely to achieve much reproductive success, as they would have to compete for territories with older, larger males. Thus, unlike young females, juvenile males presumably especially benefit from an additional year of growth prior to maturity. Of note, these findings are in accord with the accepted adaptive explanation for TSD in a fish (Conover, 1984).

Although promising, it must be recognized that studies of the adaptive significance of TSD in lizards are in their infancy. The Warner & Shine (2005) experiment, for example, focused solely on fitness-related consequences of TSD prior to reproductive maturity. Two of the substantial advantages of *A. muricatus*, in contrast to turtles and crocodylians, are its relatively short lifespan and the occurrence of congeners with GSD (Harlow, 2004). We therefore greatly anticipate reports on lifetime fitness of the individual lizards used in the Warner & Shine (2005) experiment and similar experimental studies on *Amphibolurus* species with GSD. Similarly, we strongly encourage and look forward to well-designed experiments on other lizards with relatively recent origins of TSD and on closely related taxa with GSD, as identified by phylogenetically based comparative analyses (*sensu* Janzen & Krenz, 2004). Still, much work remains as only a small fraction of lizard species have been evaluated for TSD (even in the agamids) and phylogenetic relationships within the Agamidae and in other lizard families are far from resolved.

Looking beyond the Charnov–Bull model

However, what about the other amniote taxa with TSD? In the rush to embrace the Charnov–Bull model as a general explanation for the adaptive significance of TSD in all amniotes, an important fact often appears to have been overlooked by empiricists. SDMs greatly influence primary sex ratios and selection favours those SDMs either that yield 1 : 1 primary sex ratios under standard Fisherian conditions (Fisher, 1930) or that permit faster evolution towards alternative primary sex ratios under other conditions (Bull, 1983; Freedberg & Wade, 2001). Thus, rate of sex-ratio evolution, for example, could provide a potential advantage to SDMs like TSD over GSD.

Bolstered by the absence of general empirical answers to date, we have come to believe that alternative solutions to the Charnov–Bull model may be required to identify what, if any, adaptive significance obtains for TSD in those extant taxa associated with the original evolution of this trait in amniotes. In this regard, we find advances in maternal effects theory coupled to well-developed sex-ratio theory to be a potentially fruitful approach (e.g. Freedberg & Wade, 2001). That is, by answering, ‘How does sex ratio evolve under TSD?’ we may get closer to discerning the adaptive significance and persistence of TSD in most amniotes (i.e. turtles, crocodylians and perhaps sphenodontians) (*sensu* West *et al.*, 2002).

Theoretical models (e.g. Bulmer & Bull, 1982) reveal that sex-ratio dynamics under TSD are governed by selection on, and heritable variation in, two critical factors: (1) sensitivity of embryonic sex determination to temperature and (2) maternal choice of thermal qualities of nest sites. Constant-temperature laboratory studies of the first factor have detected high heritabilities, but these estimates translate into negligible effective heritabilities when natural variation in among-nest temperatures is taken into account (e.g. Janzen, 1992). Using simple microevolutionary modelling based on the breeder’s equation, this trait would thus seem to exhibit little potential to influence sex-ratio dynamics under classic Fisherian sex-ratio selection (Janzen, 1994). Consequently, thermally based nest-site choice may be more crucial evolutionarily in maintaining TSD when sex ratio is perturbed and explaining its adaptive significance via maternal control of offspring sex ratio. It should be noted that other maternal effects not explicitly considered by theory could be relevant as well (or instead), including maternal allocation of steroid hormones to eggs (e.g. Bowden *et al.*, 2000) and nesting phenology (e.g. F.J. Janzen *et al.*, in preparation). For simplicity, we will focus only on nest-site choice.

Critical to maternal effects models is the assumption that females with TSD can and do choose nest sites based on thermally related qualities of the microhabitat. Laboratory studies of gekkotan lizards with TSD have

documented significant inheritance of (Bull *et al.*, 1988), and individual preferences for (Bragg *et al.*, 2000), nest sites with different thermal regimes. Field studies of turtles have not found definitive evidence for direct preferences for ground temperatures at oviposition (e.g. Morjan & Valenzuela, 2001). Intriguingly, however, long-term field research has noted that painted turtles (*Chrysemys picta*) possess significant, repeatable individual preferences for nest microenvironments independent of spatial philopatry on a nesting beach (Janzen & Morjan, 2001; Valenzuela & Janzen, 2001). That is, even after controlling for the geographic locations of a female’s nests, she exhibits consistent oviposition preferences for the quantity of overstory vegetation cover, an environmental variable correlated with nest temperature and offspring sex ratio (e.g. Morjan & Janzen, 2003). More recently, a substantial field heritability value for this nest-site choice ($h^2 \sim 0.30$) has been estimated using molecular markers to infer relatedness of these same free-ranging painted turtles (Gonzalez, 2004).

So, given this heritable capacity for thermally related nest-site choice in nature, do maternal effects models provide us with a satisfactory answer to the adaptive significance enigma? Admittedly, the current answer appears to be ‘No.’ As with the differential fitness assessments in long-lived amniotes with TSD (see above), recent work reveals that predictions and assumptions of two maternal effects models (Roosenburg, 1996; Reinhold, 1998) have not held up under empirical scrutiny in the field (Valenzuela & Janzen, 2001; Morjan & Janzen, 2003). Furthermore, simulation models actually suggest a surprisingly diminished role for nest-site choice in sex-ratio evolution in populations of long-lived species with TSD (Morjan, 2003). In essence, these simulations suggest that nest-site choice only permits sufficient evolutionary response of such populations to sex-ratio perturbation when climate change is relatively small ($\leq 1^\circ\text{C}$ across 100 years); climate changes $\geq 2^\circ\text{C}$ invariably led to population extinction. The results of the modelling nonetheless beg the question of how ancient clades of long-lived amniotes with TSD have successfully withstood >200 million years of substantial, often geologically swift, climate change. Indeed, experimental populations of a fish with TSD exhibited rapid evolution (<8 generations) from biased to equal sex ratio in the laboratory in response to Fisherian sex-ratio selection (Conover & Van Voorhees, 1990). The theoretical models and the historical data have yet to be reconciled in ancient clades of long-lived amniotes with TSD.

Novel experimental approaches

We nonetheless remain optimistic, being in the early stages as we are, that integrated maternal effects and sex-ratio models could provide a successful framework for elucidating the evolutionary dynamics of TSD in amniotes associated with the earliest origin of this trait. We see

potentially fruitful approaches residing in at least two complementary venues: (1) using a mix of observational and experimental studies of geographically widespread species to evaluate sex-ratio evolution in response to contemporary climatic variation and (2) employing experiments using model organisms to explore the evolution of sex ratio and SDMs under a variety of scenarios in comparison to expectations from theory. Below we provide some context regarding the value and potentially most beneficial design of these approaches, as well as tantalizing findings that motivate substantial further exploration of both avenues of research.

Examining populations of species with TSD that exhibit broad geographic distributions encompassing a variety of current climates might reveal clues to how microevolution has solved the problem of chronic, environmentally induced, biased offspring sex ratios (sensu Lagomarsino & Conover, 1993 for a fish with TSD). For example, common snapping turtles (*C. serpentina*), which have TSD, occupy aquatic habitats within North America as thermally divergent as the swamps of Louisiana (~30°N) and the waters of southern Canada (~50°N). Indeed, much of the northern range of this species was under ice ~10 000 years ago, so successful adaptation to climate in these northern areas may have been rapid. Consistent with these expectations, *C. serpentina* exhibits a strong latitudinal cline in maternal nest-site choice with respect to overstorey vegetation cover, such that nests in northern populations tend to be placed in exposed sites and those in southern populations tend to be laid in shaded sites (Ewert *et al.*, 2005). This observation suggests that nest temperatures and, thus, thermal sensitivity of embryonic sex determination should differ minimally among populations. Consequently, among other possible studies, reciprocal transplant experiments between geographically disparate populations (sensu Davis & Shaw, 2001) could provide great insight into the maternally mediated microevolution of sex ratios under TSD.

One logical approach for exploring trait evolution is to take advantage of nature's own ongoing experiments, as described above for common snapping turtles. Alternatively, researchers in many fields of biology are increasingly turning to model systems with the aim of elucidating general biological principles and mechanisms. This approach has proved fruitful in evolutionary biology as well, with the longstanding use of *Drosophila* and *Mus* species and the more recent adoption of *Arabidopsis*, *Escherichia coli*, *Caenorhabditis elegans* and other model systems. Relative ease of rearing, small organismal sizes, short generation times, well-characterized genetic and developmental pathways, and a growing understanding of genomic structure generally typify such systems.

The value of these model systems, however, is predicated on the existence of the trait of interest, among other matters, which has been an impediment to the use of any such system to explore deeply the evolution of

TSD and other SDMs. Fortunately, Hodgkin (2002) described a series of mutant strains of the model nematode *C. elegans* that apparently manifest a variety of SDMs, including several with TSD-like SDMs. [We hesitate at this early juncture to describe these strains as having true TSD (sensu Valenzuela *et al.*, 2003) until further research is conducted.] This oviparous nematode is naturally androdioecious, comprising mostly hermaphrodites, which can self-fertilize, and ~1% males, which must outcross (Hodgkin, 1987; Stewart & Phillips, 2002). The TSD-like strains possess a temperature-sensitive mutation in a sex-determining gene, such that individuals that would normally be genetic hermaphrodites can switch between a hermaphroditic or male fate depending on temperature (i.e. the normal sex chromosome system is irrelevant and is in fact rendered inoperative through the use of another mutation; Hodgkin, 2002). Offspring generally reach maturity in only a few days, creating overlapping generations as in longer-lived amniotes with TSD. These strains thus afford a tremendous opportunity to harness the manifold power of this model system to evaluate in great detail a number of key questions surrounding the evolution of environmentally sensitive SDMs that may be more elusive to address in less tractable amniote species, as well as broader questions about sex-ratio evolution (e.g. West & Sheldon, 2002).

To that end, we have begun examining aspects of SDM evolution in two strains of *C. elegans* with TSD-like sex-determining systems. Our preliminary research so far indicates that these two strains exhibit patterns of sex ratio in response to rearing temperature that correspond to patterns of TSD observed in amniotes (Fig. 3). Moreover, our initial temperature-switch experiments with these *C. elegans* strains reveal that the thermal sensitivity of sex determination occurs within a discrete range of early developmental stages (result not shown), again mimicking the embryological patterns detected in amniotes with TSD (reviewed in Janzen & Paukstis, 1991a). It should be noted further that our preliminary research over a few months to characterize aspects of apparent TSD in these lines corresponds to multiple years worth of work on amniotes with TSD (reviewed in Janzen & Paukstis, 1991a), emphasizing the flexibility and power of the *C. elegans* system. While it remains for us to cross other mutations to transform these strains from their naturally androdioecious state into dioecious strains (e.g. Stewart & Phillips, 2002; Nayak *et al.*, 2005) and to define sex-specific fitness criteria, we are excited about the potential of the *C. elegans* system to be subject to experimental evolution to test major questions about the evolutionary dynamics of TSD, including (1) the presumably central role of sex-ratio selection in molding the evolution of SDMs, (2) any sex-specific adaptive responses of TSD-like SDMs and (3) possible parallelism of alterations in genomic architecture that underlay observed phenotypic responses to sex-ratio selection in

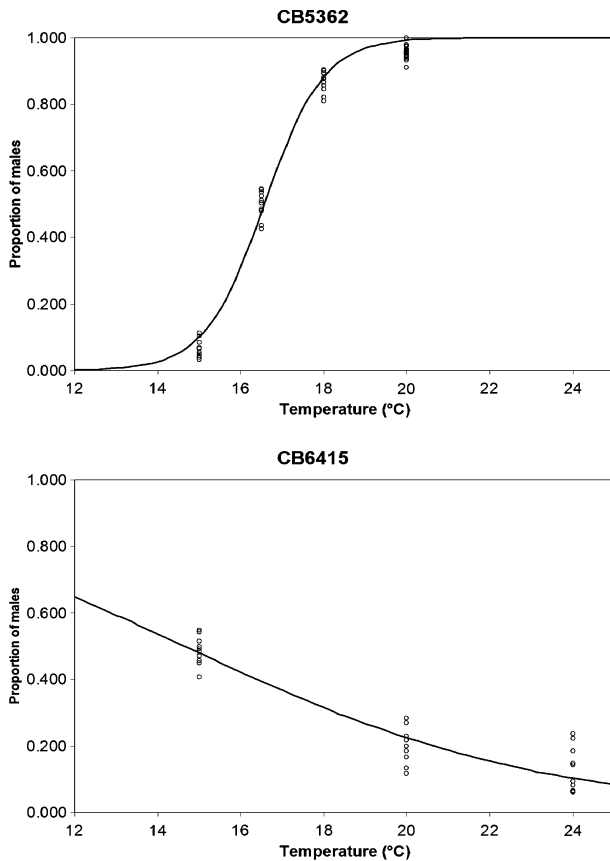


Fig. 3 Patterns of sex ratio (proportion male) in response to constant rearing temperature ($^{\circ}\text{C}$) in two strains of the nematode *Caenorhabditis elegans* with temperature-sensitive mutations in the sex determination pathway. Ten replicate plates were scored for males and hermaphrodites at each developmental temperature treatment in this preliminary work.

replicate lines of each *C. elegans* experimental strain. While these nematodes obviously are not amniote vertebrates, we believe that the results of such experiments can provide crucial tests of theory. For example, suppose experiments repeatedly reveal that TSD-like strains adaptively evolve more swiftly than GSD-like strains in response to perturbed sex ratios. If so, then researchers would need to consider strongly the possibility that sex-ratio evolution (instead of or in addition to a Charnov–Bull scenario) drives the adaptive evolution of ESD in natural systems. Consequently, the *C. elegans* system can potentially provide fresh conceptual insight into, and new directions for exploring, the natural evolution of TSD and alternative forms of ESD in other systems.

The future

In this review we have outlined what we see as key challenges in elucidating the evolution of SDMs, partic-

ularly TSD, in amniote vertebrates. Previous reviews of this topic have covered the literature and lamented the lack of progress, but not offered much in the way of prescriptions for solving the major issues. Clearly there is much crucial work yet to be done, but recent and ongoing research on key aspects of TSD both highlights the strengths and weaknesses of our theoretical and empirical backdrop and illuminates future directions for the field. Explicit phylogenetic analysis of the history of SDMs in amniotes identifies GSD as almost certainly the ancestral state of sex determination in tetrapod vertebrates, making evident that TSD in amniotes is a derived, classic case of polyphenism. Recent origins of TSD in squamates appear to validate the Charnov–Bull differential fitness approach and the predictions/directions identified by the phylogenetic analyses. Still, whether we ever identify a general explanation for the adaptive significance of this unusual SDM remains to be seen given the longstanding difficulties in successfully applying the framework of the Charnov–Bull model in other amniote taxa. Nonetheless, clearly something unique happened long ago with the ancient origination of TSD in amniotes. Because the evolution of SDMs is inextricably linked to the evolution of sex ratio, perhaps linking maternal effects and sex ratio models will provide the necessary theoretical framework for these other taxa. Intriguing new studies of microevolutionary aspects of sex ratio in turtles with TSD and the utility of experimental evolution approaches with engineered model organisms promise a bright future for ultimately elucidating the historical and current biological significance of different SDMs, particularly forms of ESD. We eagerly await the answers. If these approaches come up empty, however, we may then have to face the possibility that the initial adaptive significance of the ancient origin of TSD can no longer be determined in many, most, or all amniote clades, and that it has simply been maintained as an approximately neutral trait (Janzen & Paukstis, 1988; Girondot & Pieau, 1999).

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