Sex Chromosome Evolution in Lizards: Independent Origins and Rapid Transitions

T. Ezaz\textsuperscript{a}  S.D. Sarre\textsuperscript{a}  D. O’Meally\textsuperscript{b}  J.A. Marshall Graves\textsuperscript{b}  A. Georges\textsuperscript{a}

\textsuperscript{a}Institute for Applied Ecology, University of Canberra, and \textsuperscript{b}Research School of Biology, Australian National University, Canberra, A.C.T., Australia

Key Words
Cytogenetics · Gene mapping · Genotypic sex determination · Reptile · Squamata · Temperature-dependent sex determination

Abstract
Reptiles epitomize the variability of reproductive and sex determining modes and mechanisms among amniotes. These modes include gonochorism (separate sexes) and parthenogenesis, oviparity, viviparity, and ovoviviparity, genotypic sex determination (GSD) with male (XX/XY) and female (ZZ/ZW) heterogamety and temperature-dependent sex determination (TSD). Lizards (order Squamata, suborder Sauria) are particularly fascinating because the distribution of sex-determining mechanisms shows no clear phylogenetic segregation. This implies that there have been multiple transitions between TSD and GSD, and between XY and ZW sex chromosome systems. Approximately 1,000 species of lizards have been karyotyped and among those, fewer than 200 species have sex chromosomes, yet they display remarkable diversity in morphology and degree of degeneration. The high diversity of sex chromosomes as well as the presence of species with TSD, imply multiple and independent origins of sex chromosomes, and suggest that the mechanisms of sex determination are extremely labile in lizards. In this paper, we review the current state of knowledge of sex chromosomes in lizards and the distribution of sex determining mechanisms and sex chromosome forms within and among families. We establish for the first time an association between the occurrence of female heterogamety and TSD within lizard families, and propose mechanisms by which female heterogamety and TSD may have co-evolved. We suggest that lizard sex determination may be much more the result of an interplay between sex chromosomes and temperature than previously thought, such that the sex determination mode is influenced by the nature of heterogamety as well as temperature sensitivity and the stage of sex chromosome degeneration.

Sex chromosomes differ from autosomes in that the two members of the sex chromosome pair typically vary in morphology and gene content. They are highly specialized and appear to have evolved independently many times in vertebrates [for review see Graves, 2008]. Sex chromosomes are thought to evolve from an autosomal pair by the acquisition of a male or female-determining gene that defines a non-recombining region which is progressively extended, promoting degeneration of the sex-specific chromosome [Muller, 1914; Ohno, 1967; Charles-
worth, 1991]. This mode of sex determination is often referred to as chromosomal sex determination or genetic sex determination (GSD).

GSD vertebrates typically have either a male heterogametic (XY male/XX female) or a female heterogametic (ZZ male/ZW female) sex chromosome system. The XX/XY sex chromosome system is conserved in therian mammals, and a ZZ/ZW pair is conserved within birds and within snakes [Ohno, 1967; Graves and Shetty, 2000; Matsuda et al., 2005]. The sex chromosome pair (X and Y, or Z and W) may differ only in a restricted region (even a single locus), as is expected given their autosomal origin, or can be highly differentiated as a result of progressive degeneration due to suppression of recombination. However, vertebrate XY and ZW sex chromosomes are not homologous, suggesting independent evolution of sex chromosomes in different lineages from non-homologous ancestral autosomes [Fridolfsson et al., 1998; Nanda et al., 2000, 2002]. In some lineages, notably birds and mammals, sex chromosomes and sex determination are very stable. For example, all mammals have GSD with male heterogamety and sex is determined in therian mammals by the presence of a master sex-determining gene (e.g. SRY), on the Y chromosome [Sinclair et al., 1990]. All birds also have GSD but with female heterogamety and sex, at least in chickens, is determined by dosage of the DMRT1 gene located on the Z chromosome [Smith et al., 2009].

Many reptiles have GSD but, unlike birds or mammals, sex can also be determined by incubation temperature (temperature-dependent sex determination or TSD) independent of specific genes or chromosomes [Bull, 1983; Charnier, 1966]. In yet others, sex is determined by an interaction between environmental influences and genetic factors [Quinn et al., 2007; Radder et al., 2008]. The widespread influence of temperature on sex determination may provide a viable state from which novel master sex genes and chromosomes in reptiles can evolve [Quinn, 2008; Georges et al., 2010] and influence directly the manner in which sex chromosomes are acquired and lost. The high diversity of sex determining mechanisms seen in reptiles (e.g. XY, XXY, ZW, ZZW, TSD, genetic-environment interactions; table 1) is testament to the evolutionary lability of reptile sex determination.

There are close to 5,000 lizard species [Uetz, 2009] and they exhibit an astonishing array of reproductive and sex determining modes including gonochorism (separate sexes) and parthenogenesis, oviparity, viviparity, and ovoviviparity, GSD and TSD, and male (XX/XY) and female (ZZ/ZW) heterogamety. They are particularly fascinating because the distribution of sex-determining mechanisms shows no clear phylogenetic segregation [Janzen and Phillips, 2006; Organ and Janes, 2008; Pokorná and Kratochvíl, 2009] (table 1).

Lizards with GSD display remarkable diversity in sex chromosome differentiation, ranging from cryptic or homomorphic to highly differentiated (fig. 1). Much of this variation occurs within families, often among closely related species and even within the various races or populations of the same species. For example, the gekkonid lizard, Gehyra purpurascens, displays two Z chromosome and six W chromosome morphs, primarily as the result of centromeric inversions [Moritz, 1984] (fig. 1). Variation in the morphology of sex chromosomes among closely-related taxa, or populations of one taxon, indicate that morphological evolution of sex chromosomes, and perhaps also sex-determining mechanisms in lizards may occur relatively easily in comparison to mammals and birds.

There are substantial gaps in our knowledge of the distribution and characteristics of sex chromosomes within and among lizard families. There are data from all member species in only two families, one of which is monotypic and the other bitypic (Rhineuridae and Annielidae, respectively), and for many of these, only one animal has been examined, thereby excluding an examination for sex chromosomes (table 1). In addition, many lizards have sex microchromosomes that are difficult to identify using standard banding and staining techniques. Modern cytogenetic techniques, such as comparative genomic hybridization (CGH) that are more sensitive than G- and C-banding have enabled the characterization of sex chromosomes across a much wider range of species. This includes the identification of sex chromosomes that were cryptic because they involve microchromosomes or because they have undergone little morphological differentiation [Traut et al., 1999; Ezaz et al., 2005; Kawai et al., 2007].

Here, we review the current state of knowledge of sex chromosomes in lizards and the distribution of sex-determining mechanisms and sex chromosomal forms within and among families. We establish for the first time an association between the occurrence of female heterogamety and TSD within lizard families, and propose mechanisms by which female heterogamety and TSD may have co-evolved. We suggest that lizard sex determination may be much more the result of an interplay between sex chromosomes and temperature than previously thought, such that the sex determination mode is influenced by the nature of heterogamety, temperature sensitivity and the stage of sex chromosome degeneration.
An Astonishing Variety of Sex Chromosomes in Lizards

Karyotype information is available for at least one species from 29 of the 32 lizard families [Olmo and Signorino, 2005; Vidal and Hedges, 2009]. No species of Cadeidae, Lanthanotidae or Shinisauridae have been examined. Seven families (Iguanidae, Scincidae, Sphaerodactylidae, Pygopodidae, Dibamidae, Teiidae and Gymnophthalmidae) include species with male heterogamety (XX/XY) and 6 families (Bipedidae, Lacertidae, Teiidae, Gymnophthalmidae, Anguidae, Annielidae) species with male heterogamety (XX/XY) and 6 families (Bipedidae, Lacertidae, Teiidae, Gymnophthalmidae, Anguidae, Annielidae) species with female heterogamety (ZZ/ZW). Only 1 family (Gekkonidae) includes species with both male and female heterogamety. No sex chromosomes have been detected in the remaining 18 families (table 1).

At the level of species, approximately 913 have been karyotyped. Of these, sex chromosomes have been detected in only 181 species of which, about two thirds (115, 64%) have male heterogamety (XX/XY) and the remainder (66, 36%) female heterogamety (XX/XY) and the remainder (66, 36%) female heterogamety (XX/XY).

Data compiled from various sources including Donnellan, 1985; Zeng et al., 1997; Yonenaga-Yassuda and Rodrigues, 1999; Harlow, 2004; Andrews, 2005; Ezaz et al., 2005, 2009a; Olmo and Signorino, 2005; Yonenaga-Yassuda et al., 2005; Kawai et al., 2009; Pokorná and Kratochvíl, 2009; Gamble, 2010. TSD = Temperature dependent sex determination; NKD = number of species karyotyped; SCH = species with sex chromosomes; TNS = total number of species; PKD = proportion karyotyped; PSC = proportion with sex chromosomes.
Although the majority of lizards with sex chromosomes have XY or ZW type systems, approximately 23% have multiple sex chromosomes (table 1). Multiple sex chromosome systems, thought to have evolved via autosomal-sex chromosome fusions [Wright, 1973; King, 1977; Olmo, 1986; Odierna et al., 2001; Leache and Sites, this issue], are common in XY, but not in ZW species. Only 4 (6%) of all female heterogametic species have multiple sex chromosome systems (Z1Z1Z2Z2 male and Z1Z2W female, table 1, fig. 1) and these are confined to the family Lacertidae (3 species of Lacerta and one species of Podacris [Olmo and Signorino, 2005] (table 1). In contrast 37 (32%) of the 115 species with XY sex chromosomes have multiple sex chromosomes (X1X1X2X2 female and X1X2Y male), most of which occur in iguanidae (33/37, table 1, fig. 1).

In addition, both simple and multiple sex chromosomes have evolved within closely related species (e.g.,...
Anolis) and even different populations of the same species have been found to include multiple and simple sex chromosome systems. Intra-specific variation involving multiple XY sex chromosome systems occurs in populations of Scincella lateralis (XY/XXY, fig. 1) [Wright, 1973] and Sceloporus clarkii [Leache and Sites, this issue], while multiple ZW systems occur in Lacerta vivipera (Z1Z1Z2Z2/Z1Z2W) [Odierna et al., 2001].

A number of mechanisms have been proposed to explain the formation of heteromorphic sex chromosomes in lizards [Cole et al., 1967; King, 1981; Bickham, 1984; Olmo, 1986; Olmo et al., 1987]. Most of these are derived from observation through classical cytogenetic analysis (mainly differential staining and banding) and include peri- or para-centromeric inversion, centric fusion, heterochromatinization followed by amplification of tandem repeats, heterochromatinization followed by degeneration, and autosome-sex chromosome translocation. Rather than degeneration, as seen in the sex chromosomes of mammals and most birds and some snakes, the sex-specific chromosomes (Y or W) in many lizards are larger than their homologs, usually as a result of the tandem amplification of repetitive elements. For example, in Varanidae, the W chromosome is substantially larger than the Z chromosome in all 4 species with sex chromosomes, and is thought to have expanded by tandem amplification of heterochromatin [King, 1977] (fig. 1).

Like birds, turtles and snakes, most lizards have a karyotype composed of macrochromosomes (ranging from 2n = 10–46) and microchromosomes (ranging from 2n = 0–26) [Olmo and Signorino, 2005]. Microchromosomes have been found to be gene rich in birds with 2–3 times the number of genes than contained in macrochromosomes [Smith et al., 2000] and to have higher recombination rates [Rodionov et al., 1992]. In addition, microchromosomes are GC and CpG-rich and contain few repetitive elements [Hillier et al., 2004] and therefore, are likely to be important for generating genetic variation [Organ et al., 2008]. In contrast, very little (primarily from classical cytogenetics) is known about the origin, evolution and genomics of microchromosomes in lizards [Olmo, 2008].

Importantly, in some species of lizards, sex chromosomes have been found to be microchromosomes [Gorman and Atkins, 1966; Gorman, 1973; Bull, 1983; Ezaz et al., 2005; Ezaz et al., 2009a]. Sex microchromosomes have been found to be in most species of Iguanidae with multiple sex chromosomes, in all 4 species so far examined in Varanidae and 4 out of the 5 species examined in Agamidae [Gorman and Atkins, 1966; Gorman and Atkin,
The conclusion we draw from the studies reviewed above is that the lizard genome is dynamic at the level of sex chromosome organization. There is significant variation in the mode of sex determination and variation in the degree of heteromorphism with little regard for phylogenetic relationship or distance. Such variation occurs between families, species and even populations of one species.

**Multiple Origins of Sex Chromosomes in Lizards**

The extreme variation in sex-determining mechanisms within lizards is not typical of all reptile groups. Sex chromosomes are conserved within several groups. For example, chromosome 4 is the Z chromosome in all snakes studied to date [Becak et al., 1963, 1964; Ohno, 1967; Solari, 1993], whereas, chromosome pairs 4 or 5 are Z chromosomes in most birds [Suzuki, 1930; Ohno et al., 1964; Ohno, 1967; Solari, 1993]. Chromosome painting attested to the homology of the Z chromosome, even between the most distantly related birds [Shetty et al., 1999], and the gene content of the bird Z chromosome is also conserved [Fridolfsson et al., 1998; Stiglec et al., 2007]. So is the gene content of the snake Z between 3 distantly related species (*Python molurus*, *Elaphe quadrivirgata*, *Trimeresurus flavoviridis* [Matsubara et al., 2006]). In each lineage, the W chromosome shows homology to the Z, but is degraded by different degrees [Bull, 1980, 1983; Ohno, 1967].

The similar sizes of the snake and bird Z chromosomes were initially thought to reflect ancient homology [Ohno, 1969]. However, gene mapping showed that the bird and snake Z chromosomes are non-homologous; the bird Z is completely contained within the short arm of snake chromosome 2, and the snake Z corresponds to the bird chromosomes 2 and 27 [Matsuda et al., 2005; Matsubara et al., 2006]. Several recent studies using comparative gene mapping in snakes, a species of turtle (*Pelodiscus sinensis*), a species of gecko (*Gekko hokouensis*) and a species of dragon lizard (*Pogona vitticeps*) have shown that sex chromosomes, particularly ZW pairs, are not homologous between reptile groups [Matsuda et al., 2005; Matsubara et al., 2006; Kawai et al., 2007, 2009; Ezaz et al., 2009b; Kawagoshi et al., 2009]. This is consistent with the idea that sex chromosomes evolved many times independently in reptiles (fig. 2).

Comparative mapping between snakes, birds, the gecko *G. hokouensis* and the agamid lizard *P. vitticeps* confirms the independent origin of their sex chromosomes. Four genes (*ATP5A1, GHR, DMRT1, CHD1*) that are sex linked in the gecko *G. hokouensis* were found to be autosomal in the dragon lizard *P. vitticeps* [Ezaz et al., 2009b; Kawai et al., 2009]. Similarly, 5 snake sex-linked genes (*WAC, KLF6, TAX1BP1, RAB5A and CTNNB1*) lie on chromosome 6, and 5 chicken Z chromosome-borne genes are on chromosome 2 in *P. vitticeps* (fig. 2). Thus, the ZW pair of agamid lizard *P. vitticeps* is not homologous either to those of birds or snakes or the gecko *G. hokouensis* (fig. 2) [Ezaz et al., 2009b]. This suggests independent origins of sex chromosomes not only between 2 lizard species, but also among 2 species of lizards, 3 species of snakes and a species of turtle. The independent evolution of sex chromosomes is likely to be more common in lizards as more species are subjected to comparative study.

Lizards also show much variation within groups although, with only 6 functional sex-linked genes, and 4 sex chromosome or sex-linked markers mapped or tested
in only 5 species of lizards [Ezaz et al., 2009b; Quinn et al., 2009a, b], the degree of variation is debatable. W-specific markers from the Komodo dragon were found to be sex-linked in the Australian varanid V. rosenbergi [W. Smith, pers. comm.], suggesting the conservation of sex chromosome sequences within Varanidae. ZW-linked AFLP markers isolated from the dragon lizard, P. vitticeps were found by PCR analysis to be sex-linked in a closely related species, P. barbata. However, they were also found to be autosomal in several other species tested [Quinn et al., 2009b], suggesting the independent evolution of sex-chromosome-specific sequences within Australian agamids. Also, in a preliminary study, a sex-linked AFLP marker isolated from Bassiana duperreyi was found not to be sex-linked in a species of Tasmanian skink Niveoscincus ocellatus [Ezaz unpub.], suggesting independent origin of sex-specific sequences in skinks despite their morphologically conserved sex chromosomes [Donnellan, 1985; Hutchinson and Donnellan, 1992].

Another recent study on the comparative mapping of P. vitticeps sex chromosome-specific marker has provided evidence for the rapid evolution of non-homologous ZW sex chromosomes within Australian dragon lizards [Ezaz et al., 2009a]. Cross-species chromosome painting using a P. vitticeps sex chromosomal marker [Quinn et al., 2009b] revealed that the ZW sex microchromosomes of 3 Australian species (P. vitticeps, P. barbata and A. nobbi) were homologous, but that those of a 4th species, C. fordi, were not. This suggests at least two independent origins of ZW sex microchromosome systems in Australian agamids [Ezaz et al., 2009a]. No comparative data on the sex chromosomes in Iguanidae and Varanidae are available, so homology between the sex chromosomes of these 3 lizard groups is unknown.

These demonstrations of variation in lizard sex chromosomes, as well as the presence of species with temperature dependent sex determination, imply multiple and independent origins of sex chromosomes, and suggest that the mechanisms of sex determination are extremely labile in this group. This lability may indicate frequent transitions between modes (TSD and GSD) and mechanisms (XY and ZW) of sex determination in lizards through the evolution of novel sex chromosomes, perhaps via the acquisition of novel genes. The activation of unlinked sex-determining genes on autosomes could also be a possible mechanism for the independent origin of sex chromosomes in different lizard lineages, as proposed for the evolution of non-homologous sex chromosomes in salmonid fishes [Woram et al., 2003].

**An Ancient Reptile ZW Pair?**

Against this variation, the conservation of the ZW pair between birds and the gecko G. hokouensis is remarkable, suggesting these 2 distantly-related taxa have conserved synteny of 6 functional genes over more than 285 MYA [Rest et al., 2003; Kawai et al., 2009]. A parsimonious view of this finding is that chickens and gecko G. hokouensis retain the primitive condition of their common ancestor, with the remaining squamate clade [Vidal and Hedges, 2009] having more recently derived states, albeit, independently derived [Ezaz et al., 2009b]. However, in the context of the very great evolutionary lability of sex chromosomes in lizards, an alternate explanation presents itself. The master sex-determining genes of chickens and gecko may well be different but by chance have come to reside on homologous chromosomal regions, either by the chance capture of sex determination by genes in a syntenic region common to the bird-gecko ancestor, or through translocation. Translocation is more likely to happen in lizards, because of the apparent lack of morphologically differentiated sex chromosomes which is likely to represent nascent sex chromosomes, and such nascent sex chromosomes are known to accumulate transposable elements and have been reported in a wide range of plants and animals [Adams et al., 2000; Skakelsky et al., 2003; Charlesworth et al., 2005; Kejnovsky et al., 2008; Matsunaga, 2009].

Support for convergence in the chromosomal location of the master sex determining gene comes from the observation that it is unlikely that birds and geckos have the same master sex-determining genes. The male determining gene DMRT1 in chickens is present only on the Z chromosome and sex is determined by dosage of the DMRT1 gene product, 2 copies produce males, whereas 1 copy produces females [Smith et al., 2009]. In G. hokouensis, this gene is present in both Z and W chromosomes [Kawai et al., 2009], making it unlikely to be sex-determining in this species unless a paralog of DMRT1 is present and functions as a dominant master gene (as found in Xenopus laevis; DMW) [Yoshimoto et al., 2008], which is likely to function as a suppressor of autosomal DMRT1 dosage. Moreover, closely related species of gecko have both XY and ZW sex chromosome systems as well as TSD, and possibly sex chromosome-temperature interactions (e.g. Gekko japonicus) [Tokunaga, 1985; Gamble, 2010], indicating rapid transitions between modes of sex chromosomes as well as modes of sex determination [for review see Gamble, 2010]. The emergence of a novel sex-determining gene in geckos in the 285 million years since
their divergence from birds, and their chance residence on homologous chromosomes, would seem at least an equally plausible explanation for the homology of the sex chromosomes of these taxa, given the lability of sex chromosomes and mechanisms in other lizards. The matter is unlikely to be resolved until the genetic mechanisms of their sex determination become better known and without a comprehensive genomic analysis across the squamate phylogeny (e.g. geckos, skinks, varanids, lacertids, agamids).

Whatever the mechanism of sex determination in the gecko, the homology between the gecko and bird sex chromosomes suggests that a bird-like ZW could be ancestral for reptiles. The homology between the bird ZW and the XY complex of the platypus [Veyrunes et al., 2008] suggests that this homology might extend back to a common amniote ancestor that lived 310 MYA [Graves, 2008]. Alternatively, if ZW is the ancestral state, some of the fundamental genetic machinery of a ZW-driven sex determination and differentiation network may have been retained to varying degrees in different reptile lineages, leading to a predisposition to evolve similar sex-determining mechanisms. Thus similar sex chromosome systems may have independent origins, but are constrained in some way by their phylogenetic history.

**An Association between TSD and Female Heterogamety in Lizards**

In TSD, males and females are produced differentially according to the incubation temperatures experienced by the developing embryos. Although TSD was first described in an agamid lizard, *Agama agama* [Charnier, 1966], it is more frequent in non-squamate reptiles (in possibly all crocodilians, many turtles, tuatara) than lizards. Since Charnier's discovery, TSD has been identified unequivocally in 32 species from 6 families, most of which are from the Agamidae (13/32) and Gekkonidae (8/32, table 1) families. In addition, both GSD (4 species) and TSD (5 species, table 1) have been detected in the family Eublepharidae, although no sex chromosomes are known.

Evidence of TSD has been reported in another 9 species from the families Chamaeleonidae (2 species) Lacertidae (1 species), Scincidae (4 species), Anguidae (1 species) and Varanidae (1 species) [see Harlow, 2004; Pokorná and Kratochvíl, 2009]. However, these data are equivocal mainly because of small sample size as well as the nature and design of the incubation experiments and sex identification techniques employed [see Viets et al., 1994; Harlow, 2004; Andrews, 2005; Pokorná and Kratochvíl, 2009], so we did not include those species as having TSD in this review.

The classical view of transitions between GSD and TSD has sex captured by a temperature-sensitive element, the production of lethal or suboptimal YY or WW individuals, and the loss of these chromosomal elements from the genome through natural selection or drift [Bull, 1980, 1983]. GSD arises from TSD through the capture of sex determination by novel genes which come to influence sex and the formation of sex chromosomes [Bull, 1980, 1983; Charlesworth, 1991]. However, there is emerging evidence of interactions between GSD and TSD both in forms regarded as GSD (*Bassiana duperreyi, Pogona vitticeps*) [Quinn et al., 2007; Radder et al., 2008] and forms regarded as TSD (*Amphibolurus muricatus* and *Agama impalearis* have 50:50 sex ratios at intermediate temperatures) [Harlow and Taylor, 2000; El Mouden et al., 2001] and that sex determination should be regarded more as a continuum of states than a dichotomy of GSD and TSD [Sarre et al., 2004].

Mapping the distribution of TSD and GSD across the lizard phylogeny shows an almost haphazard distribution of the traits. However, one trend demands some attention: an association between the occurrence of TSD and the occurrence of female heterogamety within families (fig. 3). This suggests that ZW sex chromosomes evolved only in those families in which species with TSD occur. An exception is the Gekkonidae, which contains species with XY, ZW or TSD (fig. 3). Is this association an artifact, arising from limited data on the mode of sex determination in the majority of the lizard lineages? Or does the pattern arise from fundamental underlying processes we do not yet fully understand?

An association might suggest that ZW systems are more prone to coming under thermal influence, or perhaps female heterogamety is in some way more compatible with selection for TSD when thermal tendencies first arise. Alternatively, it might mean that ZW systems are more compatible with the retention of an underlying genetic predisposition (cryptic sex-linked ZW chromosomes) in TSD species [Quinn, 2008]. It has also been suggested that ZW systems in lizards act via dosage mechanisms that are more likely to evolve TSD than those systems that act via male dominance [Quinn et al., 2007], and it is gene dosage systems that are more susceptible to temperature influence than dominant gene systems.
These questions arise from an apparent association between ZW systems and TSD in the lizard phylogeny but our knowledge of the mechanisms of sex determination in lizards, and in particular, the interaction between genotype and temperature in determining sex, is as yet too rudimentary to take the ideas beyond speculation. The fundamental differences to the molecular mechanisms of sex differentiation between female homogametic and female heterogametic systems is not fully known. There is evidence that both dominant and dosage mechanisms can determine sex in ZW systems (e.g. DMRT1 dosage in birds [Smith et al., 2009]; DMW dominance in *Xenopus laevis* [Yoshimoto et al., 2008]), however, no XY dosage systems have been observed in any vertebrate. It is likely that among female heterogametic lizards, some will determine sex via dosage mechanisms as in birds, while others via dominant mechanisms, as in *X. laevis*. The dominance of a master sex-determining gene could be achieved by regulation of one or more of the influential genes in the sex differentiation pathway, in the way *SRY* interacts with *SOX3* or *SOX9* as hypothesized previously in mammals [Graves, 1998]. Alternatively, it could be achieved by inhibition of what was formerly a dosage system, in which the dosage is inhibited or knocked down by members of the same gene family as is suspected in *X. laevis* (likely DMW inhibition of DMRT1). Thus, regardless of heterogamety, sex in many lizard clades may be determined by gene dosage or gene dosage captured by a ‘dominant’ master sex gene and so generally predisposed to capture of the genetic pathway of sex differentiation by exogenous cues, such as temperature.

Further studies, particularly in gekkonid and agamid lizards, could reveal the nature of such co-evolution of ZW sex chromosomes and TSD. For example, Gekkonidae is the only family where XY, ZW and TSD exist [Gamble, 2010]. If XY and ZW sex chromosomes are found to be homologous (e.g. *Rana rugosa*) [Ogata et al., 2003], and if closely related species or populations have TSD (e.g. *Gekko japonicus, G. geko, G. hokouensis*) then identifying sex-determining genes in these species and comparative analyses of gonad differentiation pathways in XY, ZW, TSD populations (species) of geckos would reveal the true nature of co-evolution of TSD and ZW sex chromosomes and mechanisms of transitions between modes. Evidence for a relationship between TSD and ZW can also be sought in species from Agamidae, particularly Australian dragon lizards, in which these features have been reliably identified [Harlow, 2004; Ezaz et al., 2005, 2009a, b; Quinn et al., 2007, 2009b] and genomic resources are already available.

**Fig. 3.** Phylogeny of reptiles [pruned phylogenetic tree modified after Vidal and Hedges, 2009] showing apparent association between TSD and female heterogamety in lizards, suggesting frequent transitions between modes and mechanisms of sex determination in lizards via TSD and female heterogamety. MYA: Million Years Ago.

**Conclusions**

Lizards occupy a key evolutionary position in the amniote phylogeny, so understanding the origin, evolution and mechanisms of sex determination and sex chromosomes will help us to better understand the origin and evolution of sex chromosomes in other vertebrates including mammals. In particular, such studies will resolve the debate about the ancestry of sex determination, TSD, or GSD, XY or ZW, but they will also unravel how GSD and TSD co-exist and how transitions between heterogamety occur.

Fewer than 200 species of lizards have identifiable sex chromosomes, yet they display remarkable diversity in morphology and extent of degeneration. The high diversity of sex chromosomes as well as the presence of species with TSD, imply multiple and independent origins of sex chromosomes, and suggest that the mechanisms of sex determination are extremely labile in lizards. This appar-
ent lability is likely to have a significant role in rapid transitions among modes and mechanisms perhaps via female heterogamety and TSD. However, little is known about the genomics of sex chromosomes and their relationship with the plasticity of sex determination in lizards.

How sex chromosomes and temperature interact to determine sex in many lizard species is not known, although theoretical models have been proposed [Bull, 1983; Quinn 2008; Georges et al., 2010]. Thermal influence on sex reversal in 2 lizard species with highly heteromorphic sex chromosomes [Quinn et al., 2007; Radder et al., 2008] is an indication of the propensity of sex determination to be sensitive to temperature among lizards. It is quite likely that sex chromosomes will be found in lizard species that have long been regarded as classical TSD species. Therefore, interactions between sex chromosomes and temperature may be more intrinsic in lizards than previously thought, and may depend on the extent of differentiation of sex chromosomes as well as heterogamety. The apparent co-occurrence of TSD and female heterogamety may provide such a clue and perhaps represent a transitional phase between alternate modes and mechanisms.

Therefore, lizard sex determination may be much more the result of an interplay between sex chromosomes and temperature than previously thought, such that the sex determination mode is influenced by the nature of the heterogamety, temperature sensitivity and the stage of sex chromosome degeneration. Future comparative genomic analysis of sex chromosomes among closely related lizard species would appear to be one of the most important steps to understanding the origin, evolution and transitions of sex chromosomes and sex determinations. Recent advances in molecular genetics, cytogenetics and sequencing technologies promise to advance our knowledge substantially in the near future.

**Acknowledgements**

We thank Ettore Olmo for the opportunity to contribute to this special issue. Many thanks to T. Gamble for discussion about sex chromosomes and sex determination in lizards and reptiles. This work is supported by the Australian Research Council Discovery Grant (DP0881196) awarded to S.D.S., A.G. and Scott V. Edwards.

**References**


Donnellan SC: The evolution of sex chromosomes in scincid lizards: School of Biological Sciences, p. 218 (Macquarie University, 1985).


Ezaz/Sarre/O’Mealy/Graves/Georges
Lizard Sex Chromosomes


Ohno S: Sex Chromosomes and Sex-Linked Genes (Springer-Verlag, Heidelberg 1967).


Sarre SD, Georges A, Quinn A: The ends of a