Sex Chromosomes

Cytogenetic and Genome Research

Cytogenet Genome Res 2009;127:249–260
DOI: 10.1159/000300507

Sex Chromosome Evolution in Lizards: Independent Origins and Rapid Transitions

T. Ezaz[a] S.D. Sarre[a] D. O'Meally[b] J.A. Marshall Graves[b] A. Georges[a]

[a]Institute for Applied Ecology, University of Canberra, and [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-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1424–8581/09/1274–0249$26.00/0

Accessible online at:
www.karger.com/cgr

Tariq Ezaz
Institute for Applied Ecology
University of Canberra, Building 3
Canberra, A.C.T. 2601 (Australia)
Tel. + 61 2 6201 2297, Fax + 61 2 6201 5305, E-Mail Tariq.Ezaz@canberra.edu.au

worth, 1991]. This mode of sex determination is often referred to as chromosomal sex determination or genotypic 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 coevolved. 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.

Table 1. Current knowledge about the occurrence of sex chromosomes and TSD among lizard families

	XY	XXY	ZW	ZZW	Cryptic	TSD	NKD	SCH	TNS	PKD	PSC
Amphisbaenidae	0	0	0	0	0	0	26	0	159	16	0
Trogonophidae	0	0	0	0	0	0	2	0	6	33	0
Bipedidae	0	0	1	0	0	0	3	1	3	100	33
Blanidae	0	0	0	0	0	0	2	0	4	50	0
Cadeidae	0	0	0	0	0	0	0	0	1	0	0
Rhineuridae	0	0	0	0	0	0	1	0	1	100	0
Lacertidae	0	0	39	4	0	0	104	43	225	46	41
Teiidae	2	0	0	0	0	0	62	2	83	75	3
Gymnophthalmidae	5	2	0	0	0	0	22	7	175	13	32
Anguidae	0	0	0	0	0	0	1	0	16	6	0
Annielidae	0	0	0	0	0	0	2	0	2	100	0
Diploglossidae	0	0	0	0	0	0	6	0	50	12	0
Helodermatidae	0	0	0	0	0	0	1	0	2	50	0
Xenosauridae	0	0	0	0	0	0	1	0	6	17	0
Lanthanotidae	0	0	0	0	0	0	0	0	1	0	0
Varanidae	0	0	4	0	0	0	23	4	68	34	17
Shinisauridae	0	0	0	0	0	0	0	0	1	0	0
Iguanidae	36	33	0	0	0	0	249	69	700	36	28
Chamaeleonidae	0	0	1	0	1	0	50	1	178	28	2
Agamidae	0	0	5	0	9	13	93	5	380	24	5
Scincidae	30	1	0	0	0	0	118	31	1,200	10	26
Xantusiidae	0	0	0	0	0	0	12	0	29	41	0
Gerrhosauridae	0	0	0	0	0	0	12	0	34	35	0
Cordylidae	0	0	0	0	0	0	11	0	55	20	0
Sphaerodactylidae	1	0	0	0	0	0	5	1	196	3	20
Gekkonidae	2	0	11	0	0	8	75	13	794	9	17
Phyllodactylidae	0	0	1	0	0	1	7	1	109	6	14
Eublepharidae	0	0	0	0	4	5	9	0	29	31	0
Diplodactylidae	0	0	0	0	0	5	4	0	25	16	0
Carphodactylidae	0	0	0	0	0	0	6	0	28	21	0
Pygopodidae	1	1	0	0	0	0	5	2	40	13	40
Dibamidae	1	0	0	0	0	0	1	1	21	5	100

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.

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 Gymnophathalmidae) include species with male heterogamety (XX/XY) and 6 families (Bipedidae, Lacertidae, Varanidae, Chameleonidae, Agamidae and Phyllodactylidae) 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 (table 1).

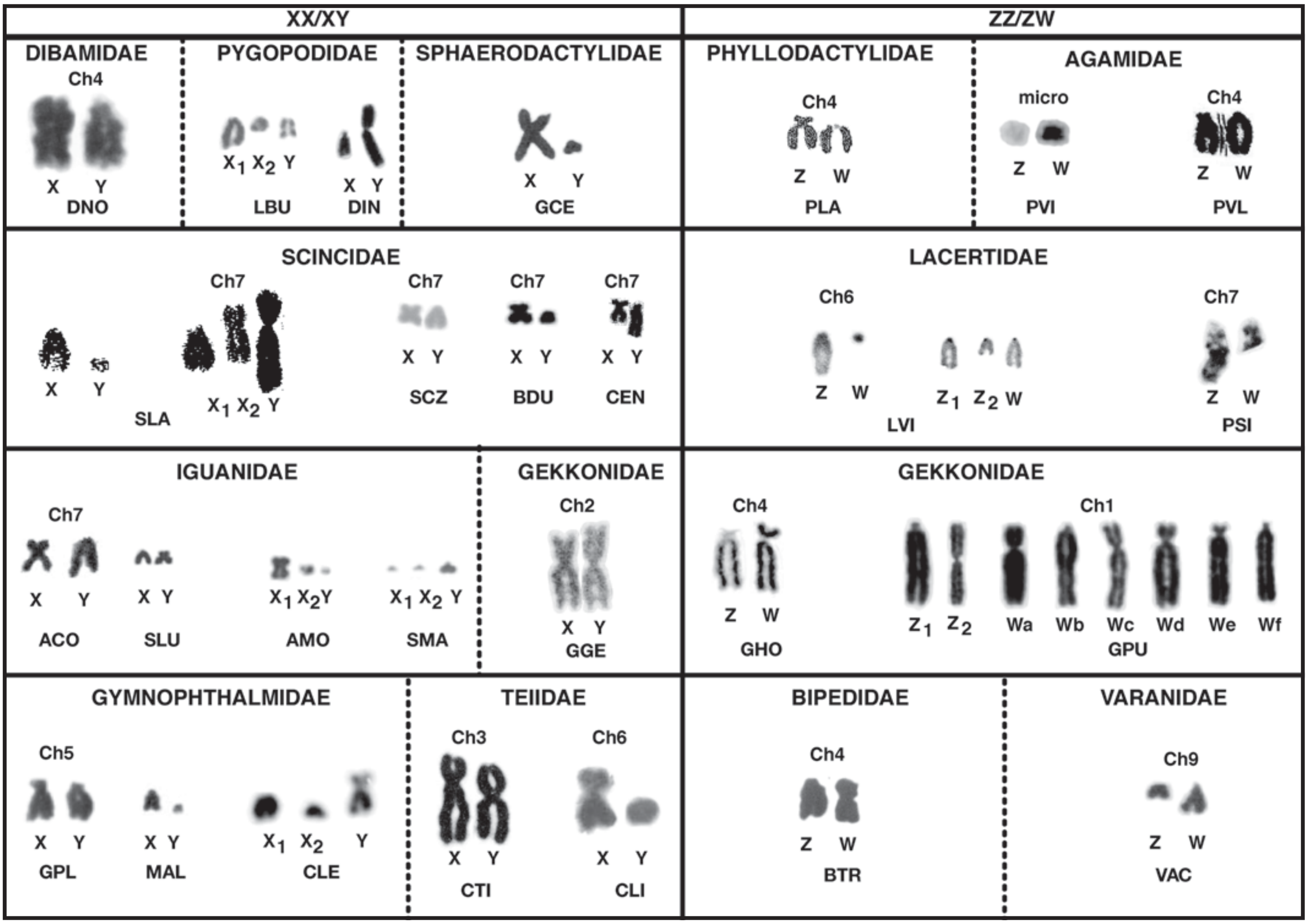

Fig. 1. A snapshot of morphological diversity of sex chromosomes in lizards. Ch: Chromosome. ACO: *Anolis conspersus* [Gorman and Aitkins, 1968], AMO: *Anolis monesis* [Gorman and Stamm, 1975], BDU: *Bassiana duperreyi* [Shine et al., 2002], BTR: *Bipes tridactylus* [Cole and Gans, 1987], CEN: *Claireascincus entrecasteauxii* [Hutchinson and Donnellan, 1992], CLE: *Calyptommatus leiolepis* [Yonenaga-Yassuda et al., 2005], CLI: *Cnemidophorus littoralis* [Peccinini-Seale et al., 2004], CTI: *Cnemidophorus tigris* [Cole et al., 1969], DIN: *Delma inornata* [King, 1990], DNO: *Dibamus novaeguineae* [Cole and Gans, 1997], GCE: *Gonatodes ceciliae* [McBee et al., 1987], GGE: *Gekko gecko* [Solleder and Schmid, 1984], GHO: *Gekko hokouensis* [Kawai et al., 2009], GPL: *Gymnophthalmus pleei* [Cole et al., 1990], GPU: *Gehyra purpurascens* [Moritz, 1984], LBU: *Lialis burtonis* [Gorman and Gress, 1970], LVI: *Lacerta vivipera* [Odierna et al., 2001], MAL: *Micrablepharus allicolus* [Yonenaga-Yassuda and Rodrigues, 1999], PLA: *Phyllodactylus lanei* [King, 1981], PSI: *Podarchis sicula* [Odierna et al., 1993], PVI: *Pogona vitticeps* [Ezaz et al., 2005], PVL: *Phrynocephalus vlangalii* [Zeng et al., 1997], SCZ: *Saproscincus czechurai* [Donnellan, 1991], SLA: *Scincella lateralis* [Wright, 1973], SLU: *Sceloporus lundelli* [Cole, 1970], SMA: *Sceloporus maculosus* [Cole, 1971], VAC: *Varanus acanthurus* [King et al., 1982].

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 autosome-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 ($Z_1Z_1Z_2Z_2$ male and Z_1Z_2W 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 ($X_1X_1X_2X_2$ female and X_1X_2Y 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* ($Z_1Z_1Z_2Z_2$/Z_1Z_2W) [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 Aitkins, 1968; King and King, 1975; King et al., 1982; Ezaz et al., 2005; Ezaz et al., 2009a]. The patterns of differentiation of these sex microchromosomes are highly variable within and among groups (fig. 1), and have evolved primarily via the accumulation and amplification of heterochromatin.

Sex microchromosomes appear to be highly labile in at least one family of lizards, with agamids exhibiting a substantial array of forms among closely related species. Four out of 5 agamid lizards whose sex chromosomes are known (*Pogona vitticeps*, *P. barbata*, *Amphibolurus nobbi* and *Ctenophorus fordi*) have ZW sex microchromosomes that are among the smallest in the complement [Ezaz et al., 2005, 2009a; Quinn et al., 2009a]. In this group, the W chromosomes are highly to moderately heterochromatic, whereas the Z chromosomes are euchromatic and can be detected only by mapping sex chromosome specific DNA sequences [Ezaz et al., 2005, 2009a] or by mapping sex chromosome specific BAC clones [Ezaz unpubl.]. Both the Z and W chromosomes are DAPI faint in all 4 species, suggesting they comprise GC rich sequences. The C-banding pattern on the W chromosomes are also variable among the 4 dragon species, ranging from fully heterochromatic in *P. barbata* and *P. vitticeps*, to highly heterochromatic in *A. nobbi* and dot-like in *C. fordi* [Ezaz et al., 2005, 2009a]. These patterns of heterochromatic variability suggest various stages of sex chromosome differentiation within closely related species.

Cryptic or homomorphic sex chromosomes are often considered to be the norm in fish, amphibians and reptiles. While this may be true for fish and amphibians where GSD is the prevalent mode of sex determination, the frequency of homomorphic sex chromosomes in reptiles is under-reported because incubation experiments have been needed to distinguish GSD species with homomorphic sex chromosomes from TSD species that lack sex chromosomes. These data are available for relatively few lizard species (table 1). Nevertheless, homomorphic sex chromosomes are certainly likely to be common in GSD lizards, as they are in fish and amphibians. High turnover of sex chromosomes, where new master sex determining genes arise on autosomes to regularly reset the progressive deterioration of the Y or W [Schartl, 2004; Volff et al., 2007], and generation of novel Y and W haplotypes by temperature induced sex reversal [Perrin, 2009], have been suggested as possible causes. Regular transitions between ZW dosage and XY dominance (or vice versa) involving the same master sex gene and associated chromosomes [Quinn, 2008] may also play a role.

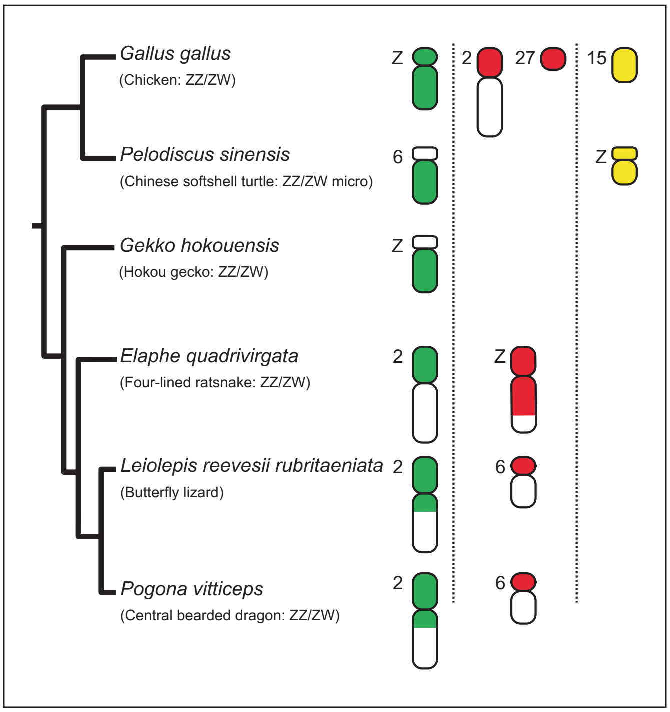

Fig. 2. Schematic representation showing the non-homology and multiple and independent origins of ZZ/ZW sex chromosome systems in reptiles based on reciprocal mapping of sex chromosomal genes from chickens, snakes and turtles. Data are summarized after [Matsuda et al., 2005; Matsubara et al., 2006; Kawai et al., 2007, 2009; Ezaz et al., 2009a, b; Kawagoshi et al., 2009; Srikulnath et al., 2009], and http://www.ensembl.org/index.html.

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; Skaletsky 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. leavis* (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. gecko*, *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.

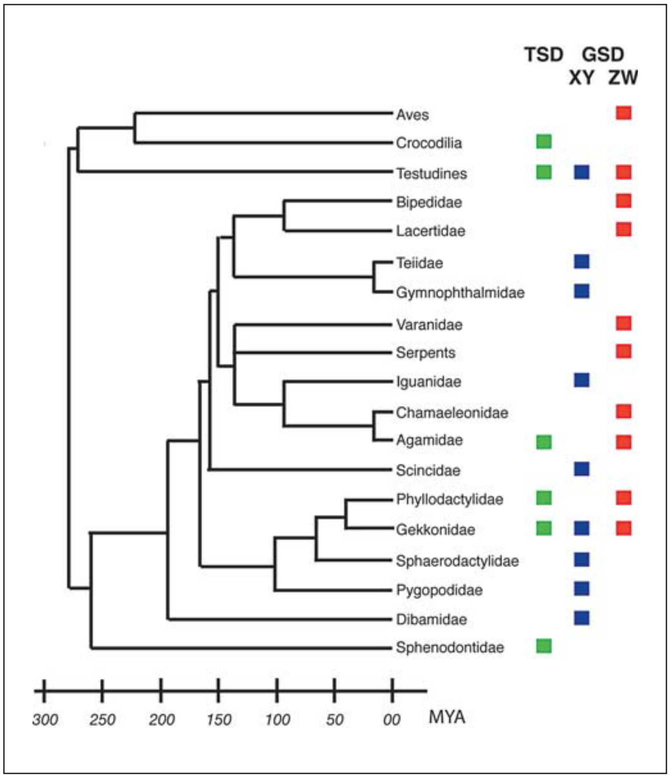

Fig. 3. Phylogeny of reptiles [pruned phylogenetic tree modified after Vidal and Hedges, 2009] showing apparent association between TSD and female heterogamety in lizards, suggesting frequent transitions between modes and mechanisms of sex determination in lizards via TSD and female heterogamety. MYA: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

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, et al: The genome sequence of *Drosophila melanogaster*. Science 287:2185–2195 (2000).

Andrews RM: Incubation temperature and sex ratio of the veiled chameleon (*Chamaeleo calyptratus*). J Herpetol 39:515–518 (2005).

Becak W, Becack ML, Nazareth HRS: Chromosomes of snakes in short term cultures of leucocytes. Am Nat 97:253–256 (1963).

Becak W, Becak ML, Nazareth HRS, Ohno S: Close karyological kinship between the reptilian suborder Serpentes and the class Aves. Chromosoma 15:606–617 (1964).

Bickham JW: Patterns and Modes of Chromosomal Evolution in Reptiles, in Sharma AK, Sharma A (eds): Chromosomes in Evolution of Eukaryotic Group, pp 13–40 (CRC Press, Boca Raton, Florida 1984).

Bull JJ: Sex determination in reptiles. Quart Rev Biol 55:3–21 (1980).

Bull JJ: Evolution of Sex Determining Mechanisms (Benjamin/Cummings, Menlo Park, California 1983).

Charlesworth B: The evolution of sex chromosomes. Science 251:1030–1033 (1991).

Charlesworth D, Charlesworth B, Marais G: Steps in the evolution of heteromorphic sex chromosomes. Heredity 95:118–128 (2005).

Charnier M: Action of temperature on the sex ratio in the *Agama agama* (Agamidae, Lacertilia) embryo. C R Seances Soc Biol Fil 160: 620–622 (1966).

Cole CJ: Karyotypes and evolution of the spinosus group of lizards in the genus *Sceloporus*. American Museum Novitates 2431:1–48 (1970).

Cole CJ: Karyotypes of the five monotypic species groups of lizards in the genus *Sceloporus*. American Museum Novitates 2450:1–18 (1971).

Cole C, Gans C: Chromosomes of *Bipes*, *Mesobaena*, and other amphisbaenians (Reptilia), with comments on their evolution. American Museum Novitates 2869:1–9 (1987).

Cole C, Gans C: The karyotype of *Dibamus novaeguineae* (Squamata). Herpetologica 53: 229–232 (1997).

Cole CJ, Lowe CH, Wright JW: Sex chromosomes in lizards. Science 155:1028–1029 (1967).

Cole CJ, Lowe CH, Wright JW: Sex chromosomes in teiid whiptail lizards (genus *Cnemidophorus*). American Museum Novitates 2395:1–14 (1969).

Cole CJ, Dessauer HC, Townsend CR, Arnold MG: Unisexual lizards of the genus *Gymnophthalmus* (Reptilia: Teiidae) in the neotropics: Genetics, orgin, and systematics. American Museum Novitates 2994:2–31 (1990).

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

Donnellan SC: Chromosomes of Australian lygosomine skinks (Lacertilia: Scincidae) ii. The genus *Lampropholis*. Genetica 83:223–234 (1991).

El Mouden EH, Znair M, Pieau C: Effects of incubation temperature on embryonic development and sex determination in the North African lizard, *Agama impalearis*. Herpetol J 11:72–77 (2001).

Ezaz T, Quinn AE, Miura I, Sarre SD, Georges A, Marshall Graves JA: The dragon lizard *Pogona vitticeps* has ZZ/ZW micro-sex chromosomes. Chromosome Res 13:763–776 (2005).

Ezaz T, Quinn AE, Sarre SD, O'Meally D, Georges A, Graves JA: Molecular marker suggests rapid changes of sex-determining mechanisms in Australian dragon lizards. Chromosome Res 17:91–98 (2009a).

Ezaz T, Moritz B, Waters PD, Graves JAM, Georges A, Sarre SD: The ZW sex microchromosomes of an Australian dragon lizard share no homology with those of other reptiles or birds. Chromosome Res 17:965–973 (2009b).

Fridolfsson AK, Cheng H, Copeland NG, Jenkins NA, Liu HC, Raudsepp T, et al: Evolution of the avian sex chromosomes from an ancestral pair of autosomes. Proc Natl Acad Sci USA 95:8147–8152 (1998).

Gamble T: A review of sex determining mechanisms in geckos (Gekkota: Squamata). Sex Dev 4 (2010).

Georges A, Ezaz T, Quinn AE, Sarre SD: Are reptiles predisposed to temperature-dependent sex determination? Sex Dev 4 (2010).

Gorman GC: The Chromosomes of the Reptilia, a Cytotaxonomic Interpretation, in Chiarelli AB, Capanna, E (eds): Cytotaxonomy and Vertebrate Evolution, pp 349–424 (Academic Press, London, New York 1973).

Gorman GC, Aitkins L: Confirmation of an X-Y sex determining mechanism in lizards (*Anolis*). Copeia 1968:159–160 (1968).

Gorman GC, Atkins L: Chromosomal heteromorphism in some male lizards of the genus *Anolis*. Am Nat 100:579–583 (1966).

Gorman GC, Gress F: Sex chromosomes of a pygopodid lizard, *Lialis burtonis*. Experientia 26:206–207 (1970).

Gorman GC, Stamm B: The *Anolis* lizards of Mona, Redonda, and La Blanquilla: chromosomes, relationships and natural history notes. J Herpetol 9:197–205 (1975).

Graves JA: Interactions between *SRY* and *SOX* genes in mammalian sex determination. Bioessays 20:264–269 (1998).

Graves JAM: Weird animal genomes and the evolution of vertebrate sex and sex chromosomes. Ann Rev Genet 42:565–586 (2008).

Graves JAM, Shetty S: The Evolution of Sex Chromosomes in Higher Vertebrates, in Clarke M (ed): Comparative Genomics, pp 153–205 (Kluwer Academic Publishers, Massachusetts 2000).

Harlow P: Temperature Dependent Sex Determination in Lizards, in Valenzuela N, Lance, VA (eds): Temperature Dependent Sex Determination in Vertebrates, pp 11–20 (Smithsonian Institution, Washington DC, 2004).

Harlow PS, Taylor JE: Reproductive ecology of the jacky dragon (*Amphibolurus muricatus*): An agamid lizard with temperature dependent sex determination. Aust Ecol 25:640–652 (2000).

Hillier LW, Miller W, Birney E, Warren W, Hardison RC, Ponting CP, et al: Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432:695–716 (2004).

Hutchinson MN, Donnellan SC: Taxonomy and genetic variation in the Australian lizards of the genus *Pseudemoia* (Scincidae: Lygosominae). J Nat Hist 26:215–264 (1992).

Janzen FJ, Phillips PC: Exploring the evolution of environmental sex determination, especially in reptiles. J Evol Biol 19:1775–1784 (2006).

Kawagoshi T, Uno Y, Matsubara K, Matsuda Y, Nishida C: The ZW micro-sex chromosomes of the chinese soft-shelled turtle (*Pelodiscus sinensis*, Trionychidae, Testudines) have the same origin as chicken chromosome 15. Cytogenet Genome Res 125:125–131 (2009).

Kawai A, Nishida-Umehara C, Ishijima J, Tsuda Y, Ota H, Matsuda Y: Different origins of bird and reptile sex chromosomes inferred from comparative mapping of chicken Z-linked genes. Cytogenet Genome Res 117: 92–102 (2007).

Kawai A, Ishijima J, Nishida C, Kosaka A, Ota H, Kohno S et al: The ZW sex chromosomes of *Gekko hokouensis* (Gekkonidae, Squamata) represent highly conserved homology with those of avian species. Chromosoma 118:43–51 (2009).

Kejnovsky E, Hobza R, Cermak T, Kubat Z, Vyskot B: The role of repetitive DNA in structure and evolution of sex chromosomes in plants. Heredity 102:533–541 (2008).

King M: The Evolution of Sex Chromosomes in Lizards, in Calaly J, Tyndale-Biscoe H (eds): Evolution and Reproduction, pp 55–60 (Australian Academy of Science, Canberra 1977).

King M: Chromosome Change and Speciation in Lizards, in Atchley W, Woodruff D (eds): Essays on Evolution and Speciation, pp 262–285 (Cambridge University Press, Cambridge 1981).

King M: Chromosomal and Immunogenetic Data: A New Perspective on the Origin of Australia's Reptiles, in Olmo E (ed): Cytogenetics of Amphibians and Reptiles (Birkhäuser, Basel 1990).

King M, King D: Chromosomal evolution in the lizard genus *Varanus* (Reptilia). Aust J Biol Sci 28:89–108 (1975).

King M, Mengden GA, King D: A pericentric-inversion polymorphism and a ZZ/ZW sex-chromosome system in *Varanus acanthurus* Boulenger analyzed by G- and C-banding and Ag staining. Genetica 58:39–45 (1982).

Matsubara K, Tarui H, Toriba M, Yamada K, Nishida-Umehara C, Agata K, et al: Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. Proc Natl Acad Sci USA 103:18190–18195 (2006).

Matsuda Y, Nishida-Umehara C, Tarui H, Kuroiwa A, Yamada K, Isobe T, et al: Highly conserved linkage homology between birds and turtles: Bird and turtle chromosomes are precise counterparts of each other. Chromosome Res 13:601–615 (2005).

Matsunaga S: Junk DNA promotes sex chromosome evolution. Heredity 102: 525–526 (2009).

McBee K, Bickham JW, Dixon JR: Male heterogamety and chromosomal variation in Caribbean geckos. J Herpetol 21:68–71 (1987).

Moritz C: The evolution of a highly variable sex chromosome in *Gehyra purpurascens* (Gekkonidae). Chromosoma 90:111–119 (1984).

Muller HJ: A gene for the fourth chromosome of *Drosophila*. J Exp Zool 17:325–336 (1914).

Nanda I, Zend-Ajusch E, Shan Z, Grutzner F, Schartl M, et al: Conserved synteny between the chicken Z sex chromosome and human chromosome 9 includes the male regulatory gene *DMRT1*: A comparative (re)view on avian sex determination. Cytogenet Cell Genet 89:67–78 (2000).

Nanda I, Haaf T, Schartl M, Schmid M, Burt DW: Comparative mapping of Z-orthologous genes in vertebrates: implications for the evolution of avian sex chromosomes. Cytogenet Genome Res 99:178–184 (2002).

Odierna G, Kupriyanova LA, Caprigilone T, Olmo E: Further data on sex chromosomes of Lacertidae and a hypothesis on their evolutionary trend. Amphibia Reptilia 14:1–11 (1993).

Odierna G, Heulin B, Guillaume C, Vogrin N, Aprea G, et al: Evolutionary and biogeographical implications of the karyological variations in the oviparous and viviparous forms of the lizard *Lacerta (Zootoca) vivipara*. Ecography 24:332–340 (2001).

Ogata M, Ohtani H, Igarashi T, Hasegawa Y, Ichikawa Y, Miura I: Change of the heterogametic sex from male to female in the frog. Genetics 164:613–620 (2003).

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

Ohno S: Evolution of sex chromosomes in mammals. Annu Rev Genet 3:495–524 (1969).

Ohno S, Stenius C, Christian LC, Becak W, Becak ML: Chromosomal uniformity in the avian subclass Carinatae. Chromosoma 15: 280–288 (1964).

Olmo E: Animal Cytogenetics 4, Chordata 3, a. Reptilia (Gebrüder Borntraeger, Stuttgart 1986).

Olmo E: Trends in the evolution of reptilian chromosomes. Integr Comp Biol 1–8 (2008).

Olmo E, Signorino G: Chromorep: A reptile chromosomes database (http://193.206.118.100/professori/chromorep.pdf, 2005).

Olmo E, Odierna G, Capriglione T: Evolution of sex-chromosomes in lacertid lizards. Chromosoma 96:33–38 (1987).

Organ C, Janes DE: Sex chromosome evolution in sauropsids. Integr Comp Biol 48:512–519 (2008).

Organ CL, Moreno RG, Edwards SV: Three tiers of genome evolution in reptiles. Integr Comp Biol 48:494–504 (2008).

Peccinini-Seale D, Rocha CFD, Almeida TMB, Araújo AFB, De Sena MA: Cytogenetics of the Brazilian whiptail lizard *Cnemidophorus littoralis* (Teiidae) from a Restinga area (Barra de Maricá) in southeastern Brazil. Braz J Biol 64:661–667 (2004).

Perrin N: Sex reversal: A fountain of youth for sex chromosomes? Evolution 63:3043–3049 (2009).

Pokorná M, Kratochvíl L: Phylogeny of sex-determining mechanisms in squamate reptiles: are sex chromosomes an evolutionary trap? Zool J Linn Soc 156:168–183 (2009).

Quinn AE: Evolution of Sex-determining Mechanisms in Reptiles: Institute for Applied Ecology, p. 196 (University of Canberra, Canberra 2008).

Quinn AE, Georges A, Sarre SD, Guarino F, Ezaz T, Graves JA: Temperature sex reversal implies sex gene dosage in a reptile. Science 316: 411 (2007).

Quinn AE, Ezaz T, Sarre SD, Graves JM, Georges A: Extension, single-locus conversion and physical mapping of sex chromosome sequences identify the Z microchromosome and pseudo-autosomal region in a dragon lizard, *Pogona vitticeps*. Heredity (2009a).

Quinn AE, Radder RS, Sarre SD, Georges A, Ezaz T, Shine R: Isolation and development of a molecular sex marker for *Bassiana duperreyi*, a lizard with XX/XY sex chromosomes and temperature-induced sex reversal. Mol Genet Genomics 281:665–672 (2009b).

Radder RS, Quinn AE, Georges A, Sarre SD, Shine R: Genetic evidence for co-occurrence of chromosomal and thermal sex-determining systems in a lizard. Biol Lett 4:176–178 (2008).

Rest JS, Ast JC, Austin CC, Waddell PJ, Tibbetts EA, et al: Molecular systematics of primary reptilian lineages and the tuatara mitochondrial genome. Mol Phylogenet Evol 29:289–297 (2003).

Rodionov A, Chelysheva LA, Solovei IV, Miakoshina IuA: Chiasma distribution in the lampbrush chromosomes of the chicken *Gallus gallus domesticus*: Hot spots of recombination and their possible role in proper disjunction of homologous chromosomes at the first meiotic division. Genetika 28:151–160 (1992).

Sarre SD, Georges A, Quinn A: The ends of a continuum: Genetic and temperature-dependent sex determination in reptiles. Bioessays 26:639–645 (2004).

Schartl M: A comparative view on sex determination in medaka. Mech Dev 121:639–645 (2004).

Shetty S, Griffin DK, Graves JA: Comparative painting reveals strong chromosome homology over 80 million years of bird evolution. Chromosome Res 7:289–295 (1999).

Shine R, Elphick MJ, Donnellan SC: Co-occurrence of multiple, supposedly incompatible modes of sex determination in a lizard population. Ecol Lett 5:486–489 (2002).

Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, et al: A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346:240–244 (1990).

Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, et al: The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 423:825–837 (2003).

Smith CA, Clifford V, Western PS, Wilcox SA, Bell KS, Sinclair AH: Cloning and expression of a *DAX1* homologue in the chicken embryo. J Mol Endocrinol 24:23–32 (2000).

Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie PG, et al: The avian Z-linked gene *DMRT1* is required for male sex determination in the chicken. Nature 461:267–271 (2009).

Solari AJ: Sex Chromosomes and Sex Determination in Vertebrates. (CRC Press, Boca Raton, FL, 1993).

Solleder E, Schmid M: XX/XY sex chromosomes in *Gekko gecko* (Sauria, Reptilia). Amphibia Reptilia 5:339–345 (1984).

Srikulnath K, Matsubara K, Uno Y, Thongpan A, Suputtitada S, et al: Karyological characterization of the butterfly lizard (*Leiolepis reevesii rubritaeniata*, Agamidae, Squamata) by molecular cytogenetic approach. Cytogenet Genome Res 125:213–223 (2009).

Stiglec R, Ezaz T, Graves JA: A new look at the evolution of avian sex chromosomes. Cytogenet Genome Res 117:103–109 (2007).

Suzuki K: On the chromosomes of the domestic fowl. Zool Mag (Japan) 42:358–359 (1930).

Tokunaga S: Temperature-dependent sex determination in *Gekko japonicus* (Gekkonidae, Reptilia). Dev Growth Differ 27:117–120 (1985).

Traut W, Sahara K, Otto TD, Marec F: Molecular differentiation of sex chromosomes probed by comparative genomic hybridization. Chromosoma 108:173–180 (1999).

Uetz P: Embl reptile database (EMBL: http://www.reptile-database.org, 2009).

Veyrunes F, Waters PD, Miethke P, Rens W, McMillan D, Alsop AE, et al.: Bird-like sex chromosomes of platypus imply recent origin of mammal sex chromosomes. Genome Res 18: 965–973 (2008).

Vidal N, Hedges SB: The molecular evolutionary tree of lizards, snakes, and amphisbaenians. C R Biol 332:129–139 (2009).

Viets BE, Ewert MA, Talent LG, Nelson CE: Sex determining mechanisms in squamate reptiles. J Exp Zool 270:45–56 (1994).

Volff JN, Nanda I, Schmid M, Schartl M: Governing sex determination in fish: regulatory putsches and ephemeral dictators. Sex Dev 1: 85–99 (2007).

Woram RA, Gharbi K, Sakamoto T, Hoyheim B, Holm LE, et al: Comparative genome analysis of the primary sex-determining locus in salmonid fishes. Genome Res 13:272–280 (2003).

Wright JW: Evolution of the X_1X_2Y sex chromosome mechanism in the scincid lizard *Scincella laterale* (Say). Chromosoma 43:101–108 (1973).

Yonenaga-Yassuda Y, Rodrigues MT: Supernumerary chromosome variation, heteromorphic sex chromosomes and banding patterns in microteiid lizards of the genus *Micrablepharus* (Squamata, Gymnophthalmidae). Chromosome Res 7:21–29 (1999).

Yonenaga-Yassuda Y, Rodrigues MT, Pellegrino KCM: Chromosomal banding patterns in the eyelid-less microteiid lizard radiation: The $X_1X_1X_2X_2$:X_1X_2Y sex chromosome system in *Calyptommatus* and the karyotypes of *Psilophthalmus* and *Tretioscincus* (Squamata, Gymnophthalmidae). Genet Mol Biol 28: 700–709 (2005).

Yoshimoto S, Okada E, Umemoto H, Tamura K, Uno Y, et al: A W-linked DM-domain gene, DM-W, participates in primary ovary development in *Xenopus laevis*. Proc Natl Acad Sci USA 105:2469–2474 (2008).

Zeng XM, Wang YZ, Liu ZJ, Fang ZL, Wu GF, Papenfuss TJ, Macey JR: Karyotypes on nine species in the genus *Phrynocephalus*, with discussion of karyotypic evolution of Chinese *Phrynocephalus*. Acta Zool Sinica 43: 399–410 (1997).