Genome Biology

REVIEW

Are homologies in vertebrate sex determination due to shared ancestry or to limited options?

Jennifer A Marshall Graves[1]* and Catherine L Peichel[2]

Abstract

The same candidate genes and the same autosomes are repeatedly used as sex chromosomes in vertebrates. Are these systems identical by descent, or are some genes or chromosomes intrinsically better at triggering the first steps of sex determination?

In all but a few vertebrates there are two sexes; females make large sessile gametes (eggs) and males make small motile gametes (sperm). Yet, despite the commonality of the endpoint, there seem to be endless ways in which the sex of an animal is determined. This is not because of differences in the program of cell differentiation, which is almost unchanged throughout vertebrates, and is governed by much the same team of genes. The chaotic variation is generated almost entirely by the diversity of factors that trigger this sex-determining pathway.

In many vertebrate lineages, the trigger for the sex-determining pathway is a gene or genes, and this type of sex determination is termed genetic sex determination (GSD). In others, an environmental cue, usually temperature, is the trigger, and this mode is called environmental (temperature) determined sex (TSD). GSD may be controlled by a male-inducing factor that defines a Y chromosome in an XX female:XY male system of male heterogamety (as in humans and other mammals), or a gene that defines a female-specific W chromosome in a ZW female:ZZ male system of female heterogamety (as in birds). In some systems, the two sex chromosomes are almost identical, differing at only one or a few loci. In others, members of the pair are highly differentiated, with a large gene-rich X (or Z) and a Y (or W) with much repetitive sequence but few active genes.

*Correspondence: jenny.graves@anu.edu.au
[1]Research School of Biology, the Australian National University, Canberra, ACT 0200, Australia
Full list of author information is available at the end of the article

Yet among this hegemony, homologous sex chromosomes, and homologous sex-determining genes, reappear again and again. One explanation for this could be that sex-determining genes that are ancestral to all vertebrates re-emerge in different lineages. An alternative hypothesis could be that there are only a few genes, on a few chromosomes, that are suited for the task of sex determination, and evolution keeps rediscovering them. To distinguish between these hypotheses, we will discuss the conserved sex-determining pathway, then the genetic triggers that activate it and the chromosomes they define, before marshalling the evidence in favor of each hypothesis. We will start by briefly describing the well-known mammalian XY system of sex determination, and compare it with the stable ZW systems in snakes and birds, and the plethora of systems in other reptiles, amphibians and fish.

Vertebrate sex-chromosome systems and their homologies

Sex determination in humans and other mammals is accomplished by a conserved XY male:XX female system. Figure 1 shows the sex-determination systems that can be found in the main vertebrate groups. All placental mammals share an almost identical large, gene-rich X chromosome, and have a degenerate Y chromosome that contains the male sex-determining gene *SRY* and paralogs of a few X-borne genes [1]. Marsupial mammals have a smaller X that is homologous to only part of the placental mammal X, and the *SRY*-containing marsupial Y is minute [1]. Monotreme mammals (platypus and echidna) have a bizarre complex of 5X and 5Y chromosomes [2], which have homology not to the XY of therian mammals (marsupial and placental mammals) but to the bird Z chromosome [3].

Sex determination in birds and snakes is also accomplished by means of sex chromosomes, but they are completely the converse of the mammalian system. Males have two copies of a gene-rich Z chromosome, and females a single copy of the Z and a small heterochromatic W chromosome. The Z has been shown by chromosome painting and gene mapping to be identical in all bird species, but is not homologous with the mammalian X

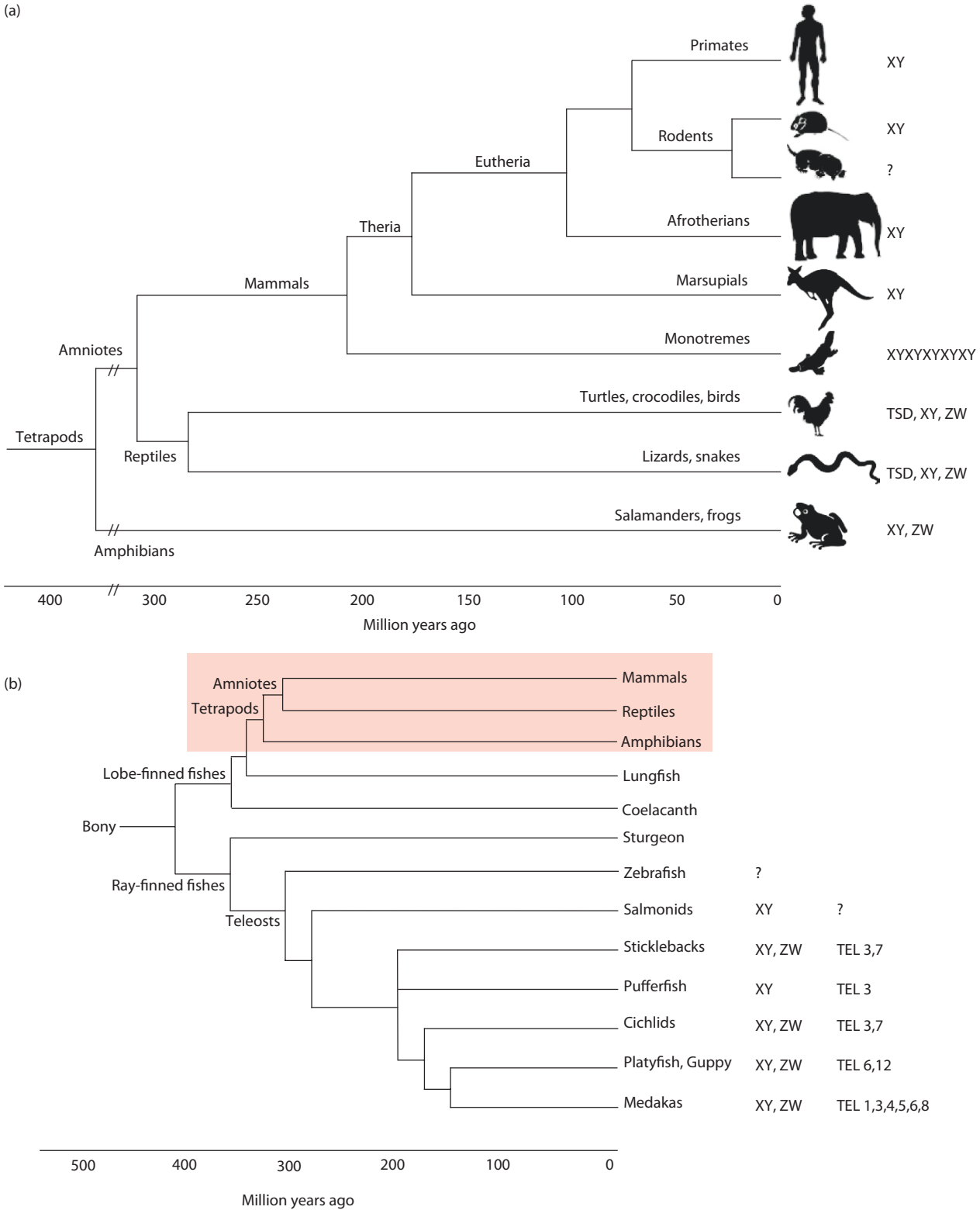

Figure 1. Phylogeny and sex-determination systems in vertebrates. (a) Tetrapods; **(b)** teleost fishes. The different sex-determination systems found in the different lineages are indicated on the right of the figure. For the fish lineages (b), homologies of identified sex chromosomes to the ancestral teleost karyotype (TEL) are shown [67]. See Table 2 for details.

[4]. The W is a degenerate version of the Z, and ranges from highly conserved in flightless ratite birds (for example, the emu) to highly differentiated in carinate birds (for example, the chicken) [4]. Snakes also have a ZZ male:ZW female system of chromosomal sex determination. As in birds, the Z chromosome is highly conserved between all snakes, whereas the W shows various degrees of degeneration [4]. The snake ZW pair superficially resembles the bird ZW pair, but comparative gene mapping shows that they share no genetic homology [5,6].

Other reptile lineages show a variety of sex-determining systems, including XY, ZW and TSD (Figure 1). Different systems are often found in the same clade [4,7] or even in the same species [8]. For example, the dragon lizard *Pogona vitticeps* has a micro-ZW system with no homology to either the snake or the bird ZW sex chromosomes, or to the mammalian XY [9]. Remarkably, though, a gekko lizard (*Gekko hokunensis*) with female heterogamety was recently discovered to have a Z chromosome with the same genes (including *DMRT1*, the bird sex-determining gene) in the same order as on the bird Z [10].

In both amphibians and fish, GSD is common (Figure 1). Morphologically distinguishable sex chromosomes are relatively rare [11,12], but studying a wider variety of species with more sophisticated cytogenetic tools, such as comparative genome hybridization, may reveal cryptic morphological differences (heteromorphy) between sex chromosomes [13,14]. Even very closely related species of fish or frogs, and even different populations within the same species, can have different sex-determination mechanisms or non-homologous sex chromosomes, as evidenced by the presence of both XY and ZW sex-chromosome systems within cichlids, sticklebacks and medaka fishes (*Oryzias*) [15-20]. In the wrinkled frog *Rana rugosa*, XY and ZW populations inhabit different Japanese islands, and there is a hybrid zone with every mixture of sex chromosomes [21,22]. Remarkably, the ZW pair of the female heterogametic population is homologous to the XY pair in the male heterogametic population [23].

Thus, outside the well-known stable mammal XY and bird and snake ZW systems, the overall picture of vertebrate sex chromosomes is one of bewildering variety (Figure 1).

The conserved sex-determination pathway

At the histological level, however, vertebrate sex determination is highly conserved. A ridge of cells on the embryonic kidney (the genital ridge) differentiates into either testis (obvious from the presence of testis cords) or ovary (obvious by the presence of large follicles surrounding developing eggs). The differentiation pathways are shown in Figure 2. The same sets of genes appear to control these alternative processes in all vertebrates, although the timing of some steps and the tissues in which the genes are expressed may differ.

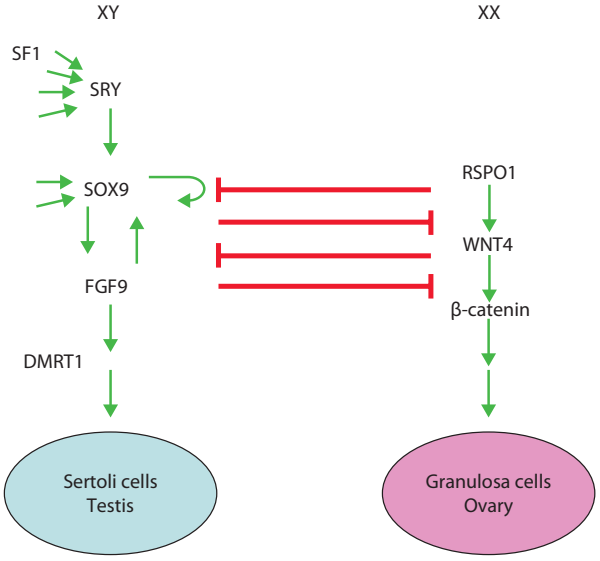

Figure 2. The antagonistic relationship between the pathways determining testis or ovary development in mammals. The male-determining pathway is shown on the left and the female pathway on the right. In males, *SRY* on the Y chromosome is activated by various upstream factors (small green arrows), including SF1, and upregulates the autosomal gene *SOX9*, which then maintains its own activity with the help of several other factors (small green arrows), including FGF9, whose expression is in turn upregulated by SOX9. FGF9 induces a cascade of downstream steps that is controlled by several genes, including *DMRT1*. These steps culminate in the differentiation of Sertoli cells, which are crucial to the development of the gonad as a testis. SOX9 and FGF9 repress RSPO1 and WNT4, which promote the development of the gonad as an ovary. In females, RSPO1 upregulates WNT4, which is accompanied by the stabilization of β-catenin, which is probably the mediator by which RSPO1 and WNT4 repress SOX9 and FGF9. Activation steps are represented by green arrows and inhibition by red bars.

To determine sex, the bipotential gonad must choose between the male or female pathway. This choice is governed by sets of genes that act to suppress each other, reinforcing one or the other cell fate [24]. In all vertebrates (TSD as well as GSD species), the autosomal gene *SOX9* seems to have a pivotal role in early testis determination. *SOX9* is present in both sexes but, in one of the first molecular events in the male pathway, it is upregulated in Sertoli cell precursors (which are essential for testis differentiation) by the primary sex-determining trigger interacting with an evolutionarily conserved sequence within SOX9 [25]. In mammals, a deficiency of *SOX9* in XY animals produces female development, and *SOX9* duplication causes male development in the absence of a Y chromosome [26].

In mammals, *SOX9* expression in males is maintained by positive feedback autoregulation, and by the action of the

Table 1. Genetic loci involved in sex determination in vertebrates

Locus	Gene name	Type of protein product
SRY	Sex-determining region on the Y chromosome	HMG-box transcription factor
SOX3	SRY-like, HMG-box-containing gene family, member 3	HMG-box transcription factor
SOX9	SRY-like, HMG-box-containing gene family, member 9	HMG-box transcription factor
SF1	Steroidogenic factor 1	Transcription factor of the steroid receptor family
FGF9	Fibroblast growth factor 9.	Secreted intercellular signal
WNT4	Wingless-type MMTV integration site family, member 4	Secreted intercellular signal
RSPO1	R-spondin-1	Secreted intercellular signal
DMRT1	Doublesex and mab-3 related transcription factor 1	Transcription factor
DMY	DMRT1 homolog on the Y in Oryzias latipes	Transcription factor
DM-W	DMRT1 homolog on the W in Xenopus laevis	Transcription factor
DGCR8	DiGeorge syndrome critical region, gene 8	microRNA biogenesis protein
RBMY	RNA-binding motif protein, Y-linked	RNA binding protein
TSPY	Testis-specific protein, Y-linked	Growth promoting factor and candidate gonadoblastoma gene.

growth factor FGF9 (*FGF9* is itself upregulated by SOX9) and several other secreted signals (Figure 2; see Table 1 for names and descriptions of the genes mentioned in the text) [27]. In males, the actions of *SOX9* and *FGF9* repress the transcriptional regulator β-catenin and the secreted intercellular signal WNT4, which promote the development of ovaries in females [24,27]. On the other hand, in females, the secreted protein R-spondin 1 (RSPO1) acts together with WNT4 to stabilize β-catenin, which then represses *SOX9* and *FGF9* [27]. Thus, there is an antagonistic relationship between testis and ovary pathways.

A single gene - *SRY* - sets off the mammalian testis-determining cascade. *SRY* was discovered on the human Y chromosome [1] and encodes a transcription factor that briefly upregulates *SOX9*, which then maintains its own expression as described above. *SRY* is mammal-specific but is the defining member of the large *SOX* gene family, members of which share the HMG-box motif that binds to DNA and bends it at a specific angle, changing chromatin structure and permitting transcription. There seems to be no comparable 'ovary-determining factor'; instead, development of the ovary is induced if the level of SOX9 is insufficient to suppress the female-promoting genes *RSPO1* and *WNT4* [24,27].

Another gene involved in vertebrate sex determination is that encoding the transcription factor DMRT1 (doublesex and mab-related transcription factor 1). *DMRT1* is the vertebrate-specific homolog of the genes *doublesex* in *Drosophila melanogaster* and *mab-3* in *Caenorhabditis elegans* (from whence it derives its name), which are involved in the downstream events of sex differentiation, rather than the initial sex-determination switch, in invertebrates [28,29]. The transcription factors encoded by these genes all share a zinc finger-like DM domain that binds DNA and regulates transcription [30,31]. Across mammals, birds, reptiles, amphibians and fish, *DMRT1* is expressed specifically in male gonads just after sex determination [28,29]. Knockdown experiments in chickens recently confirmed that *DMRT1* is the bird sex-determining gene [32]. It lies on the Z chromosome and has no allele on the heterochromatic W: the double dose of *DMRT1* is needed to form a testis in ZZ male birds, whereas the single dose in ZW females is insufficient. How *DMRT1* dosage could have a cell-autonomous effect in ZZ/ZW chimeras, as determined by Zhao *et al*. [33] is not clear. In humans, *DMRT1* is not the sex-determining trigger, but lies downstream in the sex-determining pathway. It maps to a region of chromosome 9p that is deleted in cases of XY testicular dysgenesis, and *Dmrt1* loss-of-function mutations in mice result in defects in testis development [28,29]. Thus, *DMRT1* appears to be a critical gene near the top of the sex-determination cascade in both vertebrates and invertebrates.

In addition to *SRY* and *DMRT1*, only two other vertebrate sex-determining genes are known, and remarkably they are both homologs of *DMRT1*. In the Japanese medaka fish (*Oryzias latipes*), which has an XY system, a duplicated copy of *DMRT1* (*DMY*) defines a novel Y chromosome. This novel Y chromosome is genetically the same as the X chromosome, with the addition of around 258 kb of sequence that includes the *DMY* gene. *DMY* encodes a fully functional DMRT1-type protein and has been shown to be necessary and sufficient to turn on male development [34-37]. The only known amphibian sex-determination gene, *DM-W*, is a truncated copy of *DMRT1* on the W chromosome in the frog *Xenopus laevis*. ZZ tadpoles transgenic for *DM-W* were feminized, implying that *DM-W* acts as a dominant-negative, antagonizing *DMRT1* function and repressing testis development in ZW frogs [38].

Thus, the vertebrate sex-determining pathway is extremely conserved at the molecular as well as the physiological level, but many different factors may trigger it. It has been proposed that evolutionary stability at the bottom of the sex-determination hierarchy is coupled with lability at the top [39], and the molecular and physiological conservation described above fits this notion very well. Few vertebrate sex-determining genes have been identified: *SRY* in mammals, and *DMRT1* in birds and its homologs in fish and frogs. We can deduce the deep evolutionary history of these two genes by comparing them in different vertebrate lineages in order to gain insights into the evolution of sex determination.

Evolution of sex-determining genes

When *SRY* was identified it was initially assumed to be unique to the Y chromosome. A search for *SRY* in kangaroos, however, identified a homolog on the X chromosome, termed *SOX3*. The sequence of the HMG-box in *SOX3* most closely resembled that of *SRY*, so it was suggested that *SOX3* was the ancestor of *SRY* [40]. Most other genes on the Y (for example, *RBMY*, *TSPY*), including several with male-specific roles in spermatogenesis, were subsequently found to have homologs on the X from which they had obviously evolved [1].

How did *SOX3* evolve into the testis-determining *SRY*? *SOX3* is expressed strongly in the gonads as well as in the central nervous system, at least in mouse and humans, but its deletion or duplication in human males affects fertility and intelligence rather than sex determination [41]. Its sequence similarity to *SOX9* initially prompted the suggestion that *SOX3* in a therian ancestor was originally a dosage-regulated inhibitor of *SOX9* in females, and so a null mutant of *SOX3* could have permitted activation of *SOX9* and male development. Truncation of the null *SOX3* allele was suggested to have subsequently turned it into an inhibitor of normal *SOX3*, and thus an activator of *SOX9* [42]. However, it now seems more likely that SRY interacts with steroidogenic factor 1 (SF1) to activate *SOX9* directly [43], suggesting that acquisition of a testis-determining function was due to a changed SOX3 expression pattern or to its association with different binding partners. A recent bioinformatics search of protein databases using untranslated regions of *SRY* hints that *SRY* evolved from fusion of the HMG-box of *SOX3* with another X-linked gene, *DGCR8* [44]. *SOX3* is also found on the sex chromosomes of two distantly related non-mammalian vertebrates, the frog *Rana rugosa* [23] and the fish *Oryzias dancena* (Table 2) [45]. However, it has not yet been shown to be sex-determining in either species, and is not known to be sex-linked in any other vertebrate species. Mouse knockouts indicate that *Sox3* is involved in spermatogenesis and it is a developmental regulator like *Sox1* and *Sox2*, specifying neuronal fate in fish and mammals.

DMRT1 is even more ubiquitous, being the sex-determination gene in birds, and the source of new sex-determination genes, *DMY* and *DM-W*, in fish and amphibians, respectively. *DMY* in *O. latipes* was evidently acquired about 10 million years ago, because other closely related *Oryzias* species do not share the neo-Y defined by *DMY* [46,47] and *DMRT1* is not sex-linked in any *Oryzias* species, or in any other fish with known sex chromosomes (Table 2) [48]. *DM-W* in *X. laevis* must also have been recently acquired, as close relatives (such as *X. tropicalis*) have no female-specific copy of this gene [38,49].

DMRT1 is also a candidate (albeit an unlikely one) for the sex-determining gene in monotreme mammals (platypus and echidna). It lies on one X of the platypus sex-chromosome complex, so is present in a single copy in males and two copies in females, the wrong way around for it to act to control sex by dosage. No male-specific *DMY*-like gene is detectable, so it is not clear how *DMRT1* could be implicated in monotreme sex determination.

Sex-chromosome evolution

The seemingly chaotic distribution of GSD and TSD, and XY and ZW systems described earlier, with a range of sex-chromosome differentiation between none and extreme, makes no functional sense. Rather, it is the result of evolutionary forces acting in parallel in many systems. Sex-chromosome evolution is rapid and quixotic, and plays by unique rules. We can begin to understand these rules by comparing the sex chromosomes in the different vertebrate lineages (Figures 1 and 3).

Curiously, it was comparisons of snake sex-chromosome morphology that first prompted the idea that vertebrate sex chromosomes evolved from an autosomal pair. The conservation of a large Z chromosome in all families, but morphological differences among the W chromosomes (homologous to the Z in boids such as pythons and boas; rearranged and partly heterochromatic in colubrids, the majority of snakes, and a heterochromatic rump in the evolutionarily advanced vipers) led Ohno [50] to propose that snake sex chromosomes differentiated from an original autosome pair as a sex-specific W became genetically isolated and degenerated. The same argument can be made for the bird ZW pair, in which the minimally degenerated W of ratites represents the ancient autosome. Observations of genetic homology between the bird Z and W chromosomes confirms their origin from an autosome pair [4]. Although snake and bird sex-chromosome systems are non-homologous, the bird Z is equivalent to the snake chromosome 2p (which contains *DMRT1*), and the snake Z to bird chromosome

Table 2. Known homologies of sex chromosomes in teleost fish

Species	Linkage group (LG)	Genes	*Gac*	*Tni*	*Ola*	*Dre*	TEL
Threespine stickleback *Gasterosteus aculeatus*	XX/XY - LG 19	*CYP19B* *WT1A*	LG 19 LG 19	LG 13 Sc14539	LG 6 LG 6	LG 25 LG 25	7
Ninespine stickleback *Pungitius pungitius*	XX/XY - LG 12	*PAX7* *MITFB*	LG 12 LG 12	LG 11 LG 9	LG 5 LG 7	LG11 LG 23	3
Nile tilapia *Oreochromis niloticus*	XX/XY - LG 1	*CYP19A* *WT1B*	LG 2 LG 2	LG 5 LG 5	LG 3 LG 3	LG 18 LG 18	7
Spotted tilapia *Tilapia mariae*	ZZ/ZW - LG 3	*TRP1/TYRP1A* *DMO/DMRT4*	LG 7 LG 7	Sc14681 Sc7577	LG 18 LG 18	LG 7 NF	?
Lake Malawi cichlids non-OB phenotype	XX/XY - LG 7	*IGF2* *WT1A*	LG 19 LG 19	LG 13 Sc14539	Sc1060 LG 6	LG 25 LG 25	7
Lake Malawi cichlids OB phenotype	ZZ/ZW - LG 5	*PAX7* Opsins	LG 12 LG 17	LG 11 LG 11	LG 5 LG 5	LG 11 LG 6,11	3
Tiger pufferfish *Takifugu rubripes*	XX/XY - LG 19	overall *AMHR2*	LG 17 LG 17	LG 11 LG 11	LG 5 LG 7	NA NF	3
Japanese medaka *Oryzias latipes* and *Oryzias curvinotus*	XX/XY - LG 1	*TYRP1B* *LEF1*	LG 9 LG 9	Sc13631 LG 18	LG 1 LG 1	LG 1 LG 1	4
Oryzias luzonensis	XX/XY - LG 12	SLC45A2 (b locus)	LG 14	LG 4	LG 12	LG 21	6
Oryzias mekongensis	XX/XY - LG 2	XDH POMC	LG 1	LG 3	LG 2	NF	1
Oryzias minutillus	XX/XY - LG 8	HOXBA SOX9B	LG 11 LG 11	Sc14653 LG 3	LG 8 LG 8	LG 3 LG 3	1
Oryzias dancena	XX/XY - LG10	SOX3 FGF9	LG 4 LG 4	LG 1 LG 1	LG 10 LG 10	LG 14 LG 14	5
Oryzias hubbsi	ZZ/ZW - LG 5	Opsins WNT4A	LG 17 LG 17	LG 11 LG 11	LG 5 LG 5	LG 6,11 LG 11	3
Oryzias javanicus	ZZ/ZW - LG 16	HOXAB WNT4B RSPO1	LG 10 NF LG 20	LG 8 UN NF	LG 16 LG 16 LG 16	LG 16 LG 16 LG 16	8
Guppy *Poecilia reticulata*	XX/XY - LG 12	*SLC45A2*	LG 14	LG 4	LG 12	LG 21	6
Platyfish *Xiphophorus maculatus*	XX/XY - LG 24	*MC4R*	LG 21	Sc14565	LG 20	LG 2	12
		DMRT1	LG 13	LG 12	LG 9	LG 5	6

The positions of *DMRT1* and various sex-linked genes in teleost fish species were identified by BLAT searches of the *Gasterosteus aculeatus* (*Gac*), *Tetraodon nigrividis* (*Tni*), *Oryzias latipes* (*Ola*), and *Danio rerio* (*Dre*) genomes in the Ensembl genome browser release 56. The positions of genes are indicated by linkage groups (LG), unless the gene was not found in a genome assembly (NF), in an unassembled region of the genome (UN), or on a scaffold (Sc) that has not yet been assigned to a linkage group. The homologies between different fish sex chromosomes can be seen in the column indicating its ancestral teleost protokaryotype (TEL), which was inferred on the basis of the assignment of sex-linked genes to the *Tni*, *Ola*, and *Dre* linkage groups [67]. *AMHR2*, anti-Müllerian hormone receptor, type II; *CYP19A*, cytochrome P450, family 19, paralogous gene a; *CYP19B*, cytochrome P450, family 19, paralogous gene b; *DMRT4*, doublesex and mab-3 related transcription factor 4; *HOXAB*, Hox cluster A, paralogous subgroup B; *HOXBA*, Hox cluster B, paralogous subgroup A; *IGF2*, insulin-related growth factor 2; *LEF1*, lymphoid enhancer-binding factor 1; *MITFB*, microphthalmia-associated transcription factor b; *PAX7*, paired box 7; *POMC*, proopiomelanocortin; *MC4R*, melanocortin 4 receptor; *SLC45A2*, solute carrier family 45, member 2; *TYRP1A*, tyrosinase-related protein 1; *WT1A*, Wilms tumor 1a; *WT1B*, Wilms tumor 1b; *XDH*, xanthine dehydrogenase.

2p, suggesting that they are separated by a single reciprocal translocation [5,6].

Similarly, the mammalian XY chromosome pair was suggested to have evolved from an ancestral autosomal pair by conservation of the X and degeneration of the Y to a small element retaining only a few active genes. The human X has more than 1,000 genes, but the Y retains only 45 that make unique proteins that are the relic of the Y degradation process [1]. Evidence for an autosomal origin comes from sequence homology between the human X and Y chromosomes, both within the terminal pseudoautosomal regions and between most active Y genes and their copies on the X chromosome [1]. Y degradation is rapid, fuelled by the high variation induced in the testis and the inefficiency of selection of a non-recombining entity [51]. Further degradation at this rate would lead to the extinction of the mammalian Y in a few million years [1] and indeed, several rodent lineages have already dispensed with the Y and evolved new, yet to be characterized, sex-determining genes [52-54].

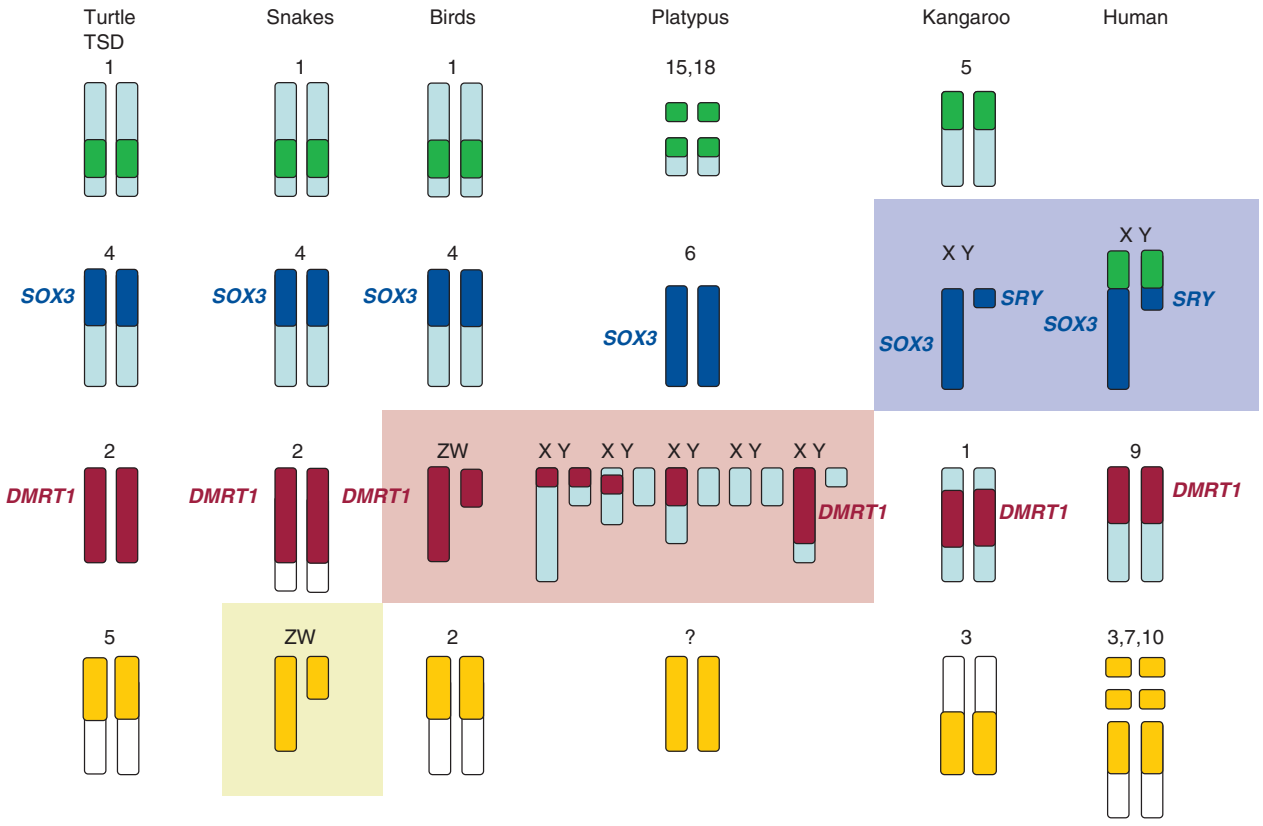

Figure 3. Orthology of sex chromosomes and autosomes in amniotes. Four conserved regions, containing orthologous genes, are represented as green, dark blue, red and yellow regions in a variety of amniotes, lying on sex chromosomes (background shaded in yellow, pink or blue) or autosomes (numbered where known). The human (eutherian) XY pair consists of the blue and green ancestral regions that are separate in reptiles and birds and in monotremes and marsupials; the blue represents the ancestral therian XY (XCR) and the green blocks an addition in eutherians (XAR or YAR). *SOX3* in the dark-blue block evolved into the mammalian sex-determining gene *SRY*. The dark-blue and the green blocks are autosomal in birds, reptiles and monotreme mammals. In birds, a different ancestral block (red), containing the sex-determining gene *DMRT1*, forms the ZW sex-chromosome pair; this gene block is also present in the ZW sex-chromosome pair in a gekko lizard (not shown), and on the sex-chromosome complex of platypus. In eutherians and marsupials, this block is autosomal. The red block is also autosomal in snakes, in which a different chromosome (yellow) has taken on a sex-determining role; the snake ZW is autosomal in all other lineages. All these genome regions are autosomal in a turtle that has temperature sex determination (TSD).

The autosomal origin of the human X and Y chromosomes was confirmed by comparative mapping, which showed that orthologs of human X-borne genes are located on autosomes in other vertebrates, and even in other groups of mammals (Figure 3). Mapping in marsupials identified a region shared by the X chromosomes of all therian mammals - the X conserved region (XCR) - and a region that is autosomal in marsupials and is proposed to have been recently added to the X chromosome in placental (eutherian) mammals (Figure 1) - the X added region (XAR) [1]. The Y chromosome, too, is made up of a comparable conserved region YCR and added region YAR; all but four of the genes on the human Y derive from the added YAR [55]. The XCR and the XAR represent two evolutionarily ancient blocks of genes that became fused before the radiation of placental mammals, approximately 105 million years ago [3]. They are present and separated in all other vertebrates; for instance, XCR genes map to chicken chromosome 4p, and XAR genes lie, in almost the same order as in humans, on the long arm of chicken chromosome 1 (Figure 3). Claims that part of the XCR represented a third ancient block that mapped elsewhere on the chicken genome [56] were based on misidentification of orthologs from the incompletely assembled chicken genome sequence [57].

In monotreme mammals, the XCR and XAR gene blocks are present and independent. However, platypus orthologs of genes from the XCR as well as the XAR are autosomal, lying on chromosome 6 (Figure 3). This implies that about 166 million years ago, the XY chromosomes of therian mammals were still an autosomal pair [3]. Thus, our sex chromosomes are relatively young, and the decline of our Y chromosome is even more precipitous than we thought [1,58].

The non-homology of the bird ZW and the therian XY systems initially suggested that they evolved independently from different autosomes. This conclusion was challenged by the finding that mammalian X and bird Z markers are syntenic in a salamander (*Ambystoma*) [59], suggesting that these regions originally formed a 'super sex chromosome' that broke up differently in the two lineages. However, this association is lacking in other vertebrates, so their synteny may be a coincidence, made more probable by the large chromosomes of *Ambystoma*.

Although these mammal, bird, and snake systems appear to be evolutionarily stable, there are many vertebrate lineages in which sex chromosomes have changed very rapidly [4]. Such switches between sex-chromosome systems are particularly enlightening. Even in mammals, there have been recent switches from the standard XY system to new systems in three rodent lineages - mole voles [52], Japanese spiny rats [53] and the Mandarin vole [54] - but we do not yet know the identity or location of the new sex-determining loci, as discussed earlier. Perhaps the best documented switch in sex chromosomes is the evolution of a neo-Y in *O. latipes* (defined by the acquisition of *DMY*), and the evolution of a neo-W in *X. laevis* (defined by *DM-W*), as discussed above. In these systems, it is proposed that acquisition of a novel male- or female-dominant sex-determining gene overrode an old system.

How such transitions can occur is still quite mysterious because we might expect that morbidity and sub-fertility of the various hybrids would select against them. However, in *Rana rugosa* (which is polymorphic for ZW and XY systems), Ogata *et al*. [21] found that hybridization creates many different sex-chromosome combinations, all of which seem to be viable. Sex-ratio biases were seen in these crosses, and the authors suggested that the transition from an XY to a ZW system resulted from selection to maintain equal sex ratios after populations hybridized. Selection for optimal sex ratios is also invoked to explain the transition between XY and ZW sex chromosomes seen in other systems, such as cichlids [60,61]. Many shifts between GSD and TSD have been documented in reptiles, and sex-chromosome switches may be facilitated in species in which an underlying genetic system interacts with a continuous variable such as temperature [62]. Examples of interactions between GSD and TSD have been documented in the dragon lizard (*P. vitticeps*) [8], the three-lined skink *Bassiana duperreyi* [63,64], and the Atlantic silverside fish *Menidia menidia* [65]. For instance, in *P. vitticeps*, ZZ males and ZW females can be recognized cytologically and by a W-specific molecular marker. Eggs incubated over a range of temperatures hatch into equal numbers of ZZ males and ZW females, but at higher temperatures all hatchlings are female, and half of these are sex reversed ZZ [8].

Despite all the evidence of rapid changes of sex chromosomes and genes in many vertebrate lineages, closer examination reveals homologous sex chromosomes, and sex-determining genes, in distantly related animals. So the questions are: is this homology the result of shared ancestry? Or is it that some genes, or some chromosomes, are particularly good at doing the job?

Is homology a relic of shared ancestry?

We will first discuss the hypothesis that homology is the result of shared ancestry. Among the diversity of vertebrate sex-determining genes and chromosomes, *DMRT1* and *SOX3* stand out because they are implicated in sex in distantly related lineages. There is evidence, too, that some sex chromosomes reappear in diverse lineages. Perhaps the most compelling case for shared ancestry is the striking homology between the bird and gekko Z chromosome and the XY chromosomes of monotreme mammals, all of which contain *DMRT1* [3,10,66]. This suggests descent from an ancestral amniote sex chromosome about 310 million years ago [58] (Figure 1a). *SOX* genes, too, turn up in distantly related lineages: as the *SRY* gene in mammals, as well as on the sex chromosomes of *Rana rugosa* [23], suggesting descent from a sex-chromosome system in an ancestral tetrapod about 400 million years ago [58].

How likely is such extraordinary sex chromosome stability? Although the mammalian, bird and snake sex-chromosome systems have been relatively stable for 166 and 80 million years, respectively, the rapid turnover of sex chromosomes within many reptiles, amphibians and fish [4] suggests that they arose independently in many different lineages. Indeed, comparing known sex chromosomes across mammals, birds, reptiles, amphibians, and fish reveals no clear ancestral vertebrate sex chromosome or gene (Figures 1 and 3). Although *DMRT1* and *SOX3* are present in distantly related vertebrates, it is difficult to see how both could be ancestral. And although *DMRT1* is implicated in sex determination in widely divergent species, fish and frog *DMRT1* homologs lie on non-homologous chromosomes. *DMRT1* is not sex-linked in therian mammals, snakes, at least one turtle, one dragon lizard, two frog, and sixteen fish species (Figure 3 and Table 2). It is thus difficult to identify an ancestral vertebrate sex chromosome.

Yet it does appear that particular chromosomes are repeatedly used as sex chromosomes in fish (Figure 1 and Table 2). We identified the locations of genes on 16 known sex chromosomes in the genomes of the three-spine stickleback (*Gasterosteus aculeatus*), green-spotted pufferfish (*Tetraodon nigroviridis*), Japanese medaka (*O. latipes*), and zebrafish (*Danio rerio*), in order to assign the sex chromosomes to the proposed ancestral teleost and vertebrate protokaryotypes [67]. The protokaryotype is the inferred karyotype of the common ancestor; that is,

the karyotype of the common ancestor of teleosts or the karyotype of the common ancestor of vertebrates. For example, the teleost protokaryotype chromosome 3/ vertebrate protokaryotype chromosome 5 appears as an XY sex chromosome in the ninespine stickleback [17,68] and the tiger pufferfish [69], and the same chromosome appears as a ZW sex chromosome in Lake Malawi cichlids [18,19] and the medaka species *O. hubbsi* [15]. Likewise, the teleost protokaryotype chromosome 7 has been independently used three times as a sex chromosome (Table 2).

Perhaps, then, the repeated appearance of homologous genes or chromosomes results from their independent reuse during sex-chromosome evolution. We shall next look at the question of whether there are limited options in the types of genes that can become sex-determination triggers or in the genomic regions that can become sex chromosomes.

Are particular genes better sex-determination triggers?

The second hypothesis set out at the beginning of this article suggests that the repeated use of the same genes for sex determination arises because they are best at doing this job. The best case for repeated use can be made for *DMRT1*, because its independent duplication, spawning novel systems (a male-dominant *DMY* in Japanese medaka and a female-dominant *DM-W* in *X. laevis*), implies that this gene is particularly suitable for such a role. Likewise, the involvement of *SOX3* homologs in XY and ZW systems in mammals and a frog suggests that this gene, too, makes a good sex-determining gene. What properties could make *DMRT1* and *SOX3* particularly adept at sex determination? These genes have two features in common. First, they encode transcription factors, and so could regulate the expression of other genes in the pathway. Second, they are related to genes that are already part of the conserved sex-determination cascade, so may be particularly favored to become master sex-determination genes [70].

SOX (*SRY*)- and *DMRT1*-related genes are clearly not the only genes that can act as triggers for sex determination. As mentioned earlier, several rodent species have lost the Y chromosome and thus lost *SRY*. In these species, therefore, there must be genes other than *SRY* that determine sex [52-54]. Testing candidate genes, including *DMRT1*, failed to find linkage to sex in these species, or in most other species examined (Figure 3 and Table 2). However, current efforts to identify the master sex-determining genes in a variety of snake, lizard, frog and fish species will clarify common features of vertebrate sex-determination genes, and permit rigorous testing of hypotheses about the types of genes that may play this important developmental role.

Do particular chromosomes make better sex chromosomes?

If vertebrate sex chromosomes are not identical by virtue of their descent from a common ancestor, how might we account for the repeated appearance of homologous sex chromosomes in diverse vertebrate lineages? Some chromosomes are surely selected because they already contain genes that are particularly well suited to a sex-determination role, such as *DMRT1* or *SOX3*. Other sex chromosomes may be defined by the acquisition of either new mutations or transposed genes, such as copies of *DMRT1*. Are particular chromosomal regions more prone to evolve other sex-determining loci? Are particular genome regions favored for such transposition events?

One compelling hypothesis that addresses these questions proposes that an autosome containing gene with sexually antagonistic effects (that is, beneficial in one sex and detrimental in the other) would be subject to selection for the spread of a linked sex-determination locus [71]. This could occur by the evolution of a novel sex-determining locus, as appears to be the case in multiple *Oryzias* species [47]. It could also occur through the transposition of an existing sex-determination locus onto another chromosome; this seems to have happened in salmonids, where markers tightly linked to the sex-determination locus are found on different chromosomes [72]. Recent studies in Lake Malawi cichlids link a pigmentation trait predicted to be under sexually antagonistic selection to a transition between an XY and a ZW sex-chromosome system [18,19]. Some rock-dwelling female cichlids have an orange-blotch color phenotype that provides camouflage but might be a disadvantage to males because it disrupts their breeding colors [19]. Consistent with the sexual antagonism theory, the gene encoding the orange-blotch phenotype lies on a W sex chromosome that is epistatic to the existing XY sex-chromosome system in Lake Malawi cichlids [18,19].

Fusions between sex chromosomes and autosomes might also link a sex-determination locus to genes with sexually antagonistic effects [73]. Y-autosome fusions have occurred in at least 25 different fish species [74], including twice independently in sticklebacks [17,20]. One Y-autosome fusion has occurred exclusively within the Japan Sea threespine stickleback population [20], males of which exhibit a unique mating behavior that is a key component of behavioral isolation from a neighboring stickleback species. This behavior maps to the neo-X chromosome created by the fusion, suggesting that sexually antagonistic selection might have driven this rearrangement to fixation [20]. Similar evolutionary forces might have been involved when an ancient ZW system underwent translocation to an ancient chromosome 2, causing a switch in the identity of the ZW chromosome pair between snakes and birds [5,6].

In mammals, it might be that the addition of the XAR (or the YAR) to the ancient mammalian XY pair added genes to the X or Y for which there was sexual antagonism [1]. The X as well as the Y chromosome is replete with genes involved in reproduction, and many of these genes are expressed in gonads in birds. Translocations between sex chromosomes and autosomes are also common in marsupials, and fusion of sex chromosomes to autosomes containing sexually antagonistic genes might provide an explanation for the bizarre translocation complex in monotreme mammals, which would be expected to be deleterious because it makes segregation of sex chromosomes into sperm difficult [2].

If sexually antagonistic selection drives the rapid turnover of sex chromosomes in fishes and other vertebrates, autosomes harboring sexually antagonistic genes could be repeatedly selected to be sex chromosomes. Interestingly, the invading ZW system of Lake Malawi cichlids involves a chromosome that shares homology with the sex chromosomes of three distantly related fish species, *Pungitius pungitius*, *Takifugu rubripes*, and *O. hubbsi* (Table 2). This chromosome also carries a number of genes that function in pigmentation pathways (*PAX7*, *WNT4A*, *MITF*) as well as opsin genes involved in color vision across these species (Table 2). Linkage between male-specific pigmentation traits and sex chromosomes also occurs in poeciliid fishes such as the guppy (*Poecilia reticulata*) and the platyfish (*Xiphophorus maculatus*) [75]. In guppies, the male-specific pigment patterns are under both natural and sexual selection [75]. Thus, it is possible that sexual antagonism for bright color patterns might contribute to the rapid turnover of sex chromosomes in fishes.

The future of research on sex determination

The availability of sequence data, and of more sophisticated molecular and cytological techniques, will revolutionize comparative studies of sex chromosomes and sex-determining genes. We can now identify cryptic sex chromosomes in even the most exotic vertebrates, obtain and map molecular markers, and deduce homologies with better-known genomes. Promising systems are those in which there has been rampant turnover within a well-defined phylogeny (for example, dragon lizards, gekkos, sticklebacks and cichlids). Such studies should identify new sex-determining genes, and enable the classification of the types of genes that might be involved.

We can also take an unbiased genome-wide approach to ask whether particular autosomes are rich in sexually antagonistic genes that could be selected for linkage with a sex-determination locus, driving the fixation of new sex chromosomes. Recently, it has been suggested that sexually dimorphic or sex-biased gene-expression patterns might reflect sexually antagonistic selection [76,77]. Sex-biased gene expression appears to be common, being found in mice, birds, flies and worms [77]. So, by analyzing genome-wide patterns of sex-biased gene expression, we could determine whether particular chromosomes contain an excess of sexually antagonistic genes, as is the case for the more ancient sex chromosomes of flies, mammals and birds [77].

Evidence that genes with potential sexually antagonistic effects lie on nascent sex chromosomes would provide compelling evidence that sexually antagonistic selection plays an important role in driving vertebrate sex-chromosome evolution and turnover. An excess of sex-biased gene expression on homologous chromosomes across a number of vertebrate systems might also explain why the same autosomes have been independently selected as sex chromosomes several times during vertebrate evolution.

In conclusion, we have provided examples of deep homology in the ZW chromosomes of birds, a gekko and monotremes, and XY chromosomes in all therian mammals. However, we also have clear examples of the independent re-use of particularly handy genes (copies of *DMRT1* and *SOX3*), and of fish chromosomes that have become sex chromosomes multiple times. Thus, the answer to the question we pose in the title is - both.

Author details
[1]Research School of Biology, the Australian National University, Canberra, ACT 0200, Australia. [2]Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA.

Published: 30 April 2010

References

1. Graves JAM: **Sex chromosome specialization and degeneration in mammals.** *Cell* 2006, **124:**901-914.
2. Grutzner F, Rens W, Tsend-Ayush E, El-Mogharbel N, O'Brien PCM, Jones RC, Ferguson-Smith MA, Graves JAM: **In the platypus a meiotic chain of ten sex chromosomes shares genes with the bird Z and mammal X chromosomes.** *Nature* 2004, **432:**913-917.
3. Veyrunes F, Waters PD, Miethke P, Rens W, McMillan D, Alsop AE, Grutzner F, Deakin J, Whittington CM, Schatzkamer K, Kremitzki CL, Graves T, Ferguson-Smith MA, Warren W, Graves JAM: **Bird-like sex chromosomes of platypus imply recent origin of mammal sex chromosomes.** *Genome Res* 2008, **18:**965-973.
4. Ezaz T, Stiglec R, Veyrunes F, Graves JAM: **Relationships between vertebrate ZW and XY sex chromosome systems.** *Curr Biol* 2006, **16:**R736-R743.
5. Matsubara K, Tarui H, Toriba M, Yamada K, Nishida-Umehara C, Agata K, Matsuda Y: **Evidence for different origin of sex chromosomes in snakes, birds, mammals and step-wise differentiation of snake sex chromosomes.** *Proc Natl Acad Sci USA* 2006, **103:**18190-18195.
6. 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* 2007, **117:**92-102.
7. Ezaz T, Quinn AE, Sarre SD, O'Meally D, Georges A, Graves JAM: **Molecular marker suggests rapid changes of sex-determining mechanisms in Australian dragon lizards.** *Chrom Res* 2009, **17:**91-98.
8. Quinn AE, Georges A, Sarre SD, Guarino F, Ezaz T, Graves JAM: **Temperature sex reversal implies sex gene dosage in a reptile.** *Science* 2007, **316:**411.
9. Ezaz T, Moritz B, Waters P, 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.** *Chrom Res* 2009, **17:**965-973.

10. Kawai A, Ishijima J, Nishida C, Kosaka A, Ota H, Kohno S, Matsuda Y: **The ZW sex chromosomes of *Gekko hokouensis* (Gekkonidae, Squamata) represent highly conserved homology with those of avian species.** *Chromosoma* 2009, **118:**43-51.
11. Devlin RH, Nagahama Y: **Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences.** *Aquaculture* 2002, **208:**191-364.
12. Schmid M, Steinlein C: **Sex chromosomes, sex-linked genes, and sex determination in the vertebrate class Amphibia.** In *Genes and Mechanisms in Vertebrate Sex Determination.* Edited by Scherer G, Schmid M. Basel: Birkhauser Verlag; 2001: 143-176.
13. Ross JA, Peichel CL: **Molecular cytogenetic evidence of rearrangements on the Y chromosome of the threespine stickleback fish.** *Genetics* 2008, **179:**2173-2182.
14. Abryamyan J, Ezaz T, Graves JAM, Koopman P: **Z and W sex chromosomes in the cane toad (*Bufo marinus*).** *Chrom Res* 2009, **17:**1015-1024.
15. Takehana Y, Naruse K, Hamaguchi S, Sakaizumi M: **Evolution of ZZ/ZW and XX/XY sex-determination systems in the closely related medaka species, *Oryzias hubbsi* and *O. dancena*.** *Chromosoma* 2007, **116:**463-470.
16. Cnaani A, Lee B-Y, Zilberman N, Ozouf-Coastaz C, Hulata G, Ron M, D'Hont A, Baroiller JF, D'Cotta H, Denpan DJ, Tomasino E, Coutanceau J-P, Pepey E, Shirak A, Kocher T: **Genetics of sex determination in tilapiine species.** *Sex Dev* 2008, **2:**43-54.
17. Ross JA, Urton JR, Boland J, Shapiro MD, Peichel CL: **Turnover of sex chromosomes in the stickleback fishes (Gasterosteidae).** *PLoS Genet* 2009, **5:**e1000391.
18. Ser JR, Roberts RB, Kocher TD: **Multiple interacting loci control sex determination in Lake Malawi cichlid fish.** *Evolution* 2010, **64:**486-501.
19. Roberts RB, Ser JR, Kocher TD: **Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi cichlid fishes.** *Science* 2009, **326:**998-1001.
20. Kitano J, Ross JA, Mori S, Kume M, Jones FC, Chan YF, Absher DM, Grimwood J, Schmutz J, Myers RM, Kingsley DM, Peichel CL: **A role for a neo-sex chromosome in stickleback speciation.** *Nature* 2009, **461:**1079-1083.
21. 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* 2003, **164:**613-620.
22. Ogata M, Hasegawa Y, Ohtani H, Mineyama M, Miura I: **The ZZ/ZW sex-determining mechanism originated twice and independently during evolution of the frog, *Rana rugosa*.** *Heredity* 2008, **100:**92-99.
23. Uno Y, Nishida C, Oshima Y, Yokoyama S, Miura I, Matsuda Y, Nakamura M: **Comparative chromosome mapping of sex-linked genes and identification of sex chromosomal rearrangements in the Japanese wrinkled frog (*Rana rugosa*, Ranidae) with ZW and XY sex chromosome systems.** *Chrom Res* 2008, **16:**637-647.
24. Kim Y, Capel B: **Balancing the bipotential gonad between alternative organ fates: a new perspective on an old problem.** *Dev Dyn* 2006, **235:**2292-2300.
25. Bagheri-Fam S, Sinclair A, Koopman P, Harley V: **Conserved regulatory modules in the Sox9 testis-specific enhancer predict roles for SOX, TCF/LEF, DMRT, and GATA proteins in vertebrate sex determination.** *Int J Biochem Cell Biol* 2010, **42:**472-477.
26. Kobayashi A, Chang H, Chaboissier MC, Schedl A, Behringer RR: ***Sox9* in testis determination.** *Ann NY Acad Sci* 2005, **1061:**9-17.
27. Nef S, Vasalli J-D: **Complementary pathways in mammalian female sex determination.** *J Biol* 2009, **8:**74.
28. Ferguson-Smith M: **The evolution of sex chromosomes and sex determination in vertebrates and the key role of *DMRT1*.** *Sex Dev* 2007, **1:**2-11.
29. Hong CS, Park B-Y, Saint-Jeannet J-P: **The function of *Dmrt* genes in vertebrate development: it is not just about sex.** *Dev Biol* 2007, **310:**1-9.
30. Yi W, Zarkower D: **Similarity of DNA binding and transcriptional regulation by *Caenorhabditis elegans* MAB-3 and *Drosophila melanogaster* DSX suggests conservation of sex determining mechanisms.** *Development* 1999, **126:**873-881.
31. Murphy MW, Zarkower D, Bardwell VJ: **Vertebrate DM domain proteins bind similar DNA sequences and can heterodimerize on DNA.** *BMC Mol Biol* 2007, **8:**58.
32. Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie PG, Doran TJ, Sinclair AH: **The avian Z-linked gene *DMRT1* is required for male sex determination in the chicken.** *Nature* 2009, **461:**267-271.
33. Zhao D, McBride D, Nandi S, McQueen HA, McGrew MJ, Hocking PM, Lewis PD, Sang HM, Clinton M: **Somatic sex identity is cell autonomous in the chicken**. *Nature* 2010, **464:**237-242.
34. Matsuda M, Nagahama Y, Shinomiya A, Sato T, Matsuda C, Kobayashi T, Morrey CE, Shibata N, Asakawa S, Shimizu N, Hori H, Hamaguchi S, Sakaizumi M: ***DMY* is a Y-specific DM-domain gene required for male development in the medaka fish**. *Nature* 2002, **417**:559-563.
35. Nanda I, Kondo M, Hornung U, Asakawa S, Winkler C, Shimizu A, Shan Z, Haaf T, Shimizu N, Shima A, Schmid M, Schartl M: **A duplicated copy of *DMRT1* in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes*.** *Proc Natl Acad Sci USA* 2002, **99:**11778-11783.
36. Kondo M, Hornung U, Nanda I, Imai S, Sasaki T, Shimizu A, Asakawa S, Hori H, Schmid M, Shimizu N, Schartl M: **Genomic organization of the sex-determining and adjacent regions of the sex chromosomes of medaka.** *Genome Res* 2006, **16:**815-826.
37. Matsuda M, Shinomiya A, Kinoshita M, Suzuki A, Kobayashi T, Paul-Prasanth B, Lau E, Hamaguchi S, Sakaizumi M, Nagahama Y: ***DMY* gene induces male development in genetically female (XX) medaka fish.** *Proc Natl Acad Sci USA* 2007, **104:**3865-3870.
38. Yoshimoto S, Okada E, Umemoto H, Tamura K, Uno Y, Nishida-Umehara C, Matsuda Y, Takamatsu N, Shiba T, Ito M: **A W-linked DM-domain gene, *DM-W*, participates in primary ovary development in *Xenopus laevis*.** *Proc Natl Acad Sci USA* 2008, **105:**2469-2474.
39. Wilkins AS: **Moving up the hierarchy: A hypothesis on the evolution of a genetic sex determination pathway.** *BioEssays* 1995, **17:**71-77.
40. Foster JW, Graves JAM: **An *SRY*-related sequence on the marsupial X chromosome: implications for the evolution of the mammalian testis-determining gene.** *Proc Natl Acad Sci USA* 1994, **91:**1927-1931.
41. Weiss J, Meeks JJ, Hurley L, Raverot G, Frasseto A, Jameson JL: ***Sox3* is required for gonadal function, but not sex determination, in males and females.** *Mol Cell Biol* 2003, **23:**8084-8091.
42. Graves JAM: **The rise and fall of *SRY*.** *Trends Genet* 2002, **18:**259-264.
43. Sekido R, Lovell-Badge R: **Sex determination involves synergistic action of *SRY* and *SF1* on a specific *Sox9* enhancer.** *Nature* 2008, **453:**930-934.
44. Sato Y, Shinka T, Sakamoto K, Ewis AA, Nakahori Y: **The male-determining gene *SRY* is a hybrid of *DGCR8* and *SOX3*, and is regulated by the transcription factor *CP2*.** *Mol Cell Biochem* 2010, **337:**267-275.
45. Takehana Y, Demiyah D, Naruse K, Hamaguchi S, Sakaizumi M: **Evolution of different Y chromosomes in two medaka species, *Oryzias dancena* and *O. latipes*.** *Genetics* 2007, **175:**1335-1340.
46. Kondo M, Nanda I, Hornung U, Schmid M, Schartl M: **Evolutionary origin of the medaka Y chromosome.** *Curr Biol* 2004, **14:**1664-1669.
47. Tanaka K, Takehana Y, Naruse K, Hamaguchi S, Sakaizumi M: **Evidence for different origins of sex chromosomes in closely related *Oryzias* fishes: substitution of the master sex-determining gene.** *Genetics* 2007, **177:**2075-2081.
48. Kondo M, Nanda I, Hornung U, Asakawa S, Shimizu N, Mitani H, Schmid M, Shima A, Schartl M: **Absence of the candidate male sex-determining gene *dmrt1b(Y)* of medaka from other fish species.** *Curr Biol* 2003, **13:**416-420.
49. Uno Y, Nishida C, Yoshimoto S, Ito M, Oshima Y, Yokoyama S, Nakamura M, Matsuda Y: **Diversity in the origins of sex chromosomes in anurans inferred from comparative mapping of sexual differentiation genes for three species of the Raninae and Xenopodinae.** *Chrom Res* 2008, **16:**999-1011.
50. Ohno S: *Sex Chromosomes and Sex-linked Genes.* Berlin: Springer-Verlag; 1967.
51. Charlesworth B, Charlesworth D: **The degeneration of Y chromosomes.** *Philos Trans R Soc Lond B Biol Sci* 2000, **355:**1563-1572.
52. Just W, Rau W, Vogel W, Akhverdian M, Fredga K, Graves JAM, Lyapunova E: **Absence of *Sry* in species of the vole *Ellobius*.** *Nat Genet* 1995, **11:**117-118.
53. Sutou S, Mitsui Y, Tsuchiya K: **Sex determination without the Y chromosome in two Japanese rodents *Tokudaia osimensis osimensis* and *Tokudaia osimensis* spp.** *Mamm Genome* 2001, **12:**17-21.
54. Chen Y, Dong Y, Xiang X, Zhang X, Zhu B: **Sex determination of *Microtus mandarinus mandarinus* is independent of *Sry* gene.** *Mamm Genome* 2008, **19:**61-68.
55. Waters PD, Kirby PJ and Graves JAM: **Assignment of the SMARCF1 gene to tammar wallaby chromosome 5q by fluorescence in situ hybridization.** *Cytogenet Cell Genet* 2001, **93:**315-316.
56. Kohn M, Kehrer-Sawatzki H, Vogel W, Graves JAM, Hameister H: **Wide genome comparisons reveal the origins of the human X chromosome.** *Trends Genet* 2004, **20:**598-603.
57. Delbridge ML, Patel HR, Waters PD, McMillan DA, Graves JAM: **Does the human X contain a third evolutionary block? Origin of genes on human**

Xp11 and Xq28. *Genome Res* 2009, **19:**1350-1360.
58. Graves JAM: **Weird animal genomes and the evolution of vertebrate sex and sex chromosomes.** *Annu Rev Genet* 2008, **42:**565-586.
59. Smith JJ, Voss SR: **Bird and mammal sex-chromosome orthologs map to the same autosomal region in a salamander (*Ambystoma*).** *Genetics* 2007, **177:**607-613.
60. Lande R, Seehausen O, van Alphen JJM: **Mechanisms of rapid sympatric speciation by sex reversal and sexual selection in cichlid fish.** *Genetica* 2001, **112-113:**435-443.
61. Kocher TD: **Adaptive evolution and explosive speciation: the cichlid fish model.** *Nat Rev Genet* 2004, **5:**288-298.
62. Sarre SD, Georges A, Quinn A: **The ends of a continuum: genetic and temperature-dependent sex determination in reptiles.** *BioEssays* 2004, **26:**639-645.
63. Shine R, Elphick MJ, Donnellan S: **Co-occurence of multiple, supposedly incompatible modes of sex determination in a lizard population.** *Ecol Lett* 2002, **5:**486-489.
64. Radder RS, Quinn AE, Georges A, Sarre SD, Shine R: **Genetic evidence for co-occurence of chromosomal and thermal sex-determining systems in a lizard.** *Biol Lett* 2008, **4:**176-178.
65. Conover DO, Kynard BE: **Environmental sex determination: interaction of temperature and genotype in a fish.** *Science* 1981, **213:**577-579.
66. Rens W, O'Brien PCM, Grutzner F, Clarke O, Graphodatskaya D, Tsend-Ayush E, Trifonov VA, Skelton H, Wallis MC, Johnston S, Veyrunes F, Graves JAM, Ferguson-Smith MA: **The multiple sex chromosomes of platypus and echidna are not completely identical and several share homology with the avian Z.** *Genome Biol* 2007, **8:**R243.
67. Kohn M, Hogel J, Vogel W, Minich P, Kehrer-Sawatzki H, Graves JAM, Hameister H: **Reconstruction of a 450-My-old ancestral vertebrate protokaryotype.** *Trends Genet* 2006, **22:**203-210.
68. Shapiro MD, Summers BR, Balabhadra S, Miller AL, Cunningham CB, Aldenhoven JT, Bell MA, Kingsley DM: **The genetic architecture of skeletal convergence and sex determination in ninespine sticklebacks.** *Curr Biol* 2009, **19:**1140-1145.
69. Kikuchi K, Kai W, Hosokawa A, Mizuno N, Suetake H, Asahina K, Suzuki Y: **The sex-determining locus in the tiger pufferfish, *Takifugu rubripes*.** *Genetics* 2007, **175:**2039-2042.
70. Pomiankowski A, Nöthiger R, Wilkins A: **The evolution of the *Drosophila* sex-determination pathway.** *Genetics* 2004, **166:**1761-1773.
71. van Doorn GS, Kirkpatrick M: **Turnover of sex chromosomes induced by genetic conflict.** *Nature* 2007, **449:**909-912.
72. Woram RA, Gharbi K, Sakamoto T, Hoyheim B, Holm L, Naish K, McGowan C, Ferguson MM, Phillips RB, Stein J, Guyomard R, Cairney M, Taggart JB, Powell R, Davidson W, Danzmann RG: **Comparative genome analysis of the primary sex-determining locus in salmonid fishes.** *Genome Res* 2003, **13:**272-280.
73. Charlesworth D, Charlesworth B: **Sex differences in fitness and selection for centric fusions between sex-chromosomes and autosomes.** *Genet Res* 1980, **35:**205-214.
74. Ueno K, Takai A: **Multiple sex chromosome system of X1X1X2X2/X1X2Y type in lutjanid fish, *Lutjanus quinquelineatus* (Perciformes).** *Genetica* 2008, **132:**35-41.
75. Lindholm A, Breden F: **Sex chromosomes and sexual selection in poeciliid fishes.** *Am Nat* 2002, **160:**S214-S224.
76. Connallan T, Knowles LL: **Intergenomic conflict revealed by patterns of sex-biased gene expression.** *Trends Genet* 2005, **21:**495-499.
77. Ellegren H, Parsch J: **The evolution of sex-biased genes and sex-biased gene expression.** *Nat Rev Genet* 2007, **8:**689-698.

doi:10.1186/gb-2010-11-4-205
Cite this article as: Marshall Graves JA, Peichel CL: **Are homologies in vertebrate sex determination due to shared ancestry or to limited options?** *Genome Biology* 2010, **11:**205.