Relationships between Vertebrate ZW and XY Sex Chromosome Systems

Review

Tariq Ezaz, Rami Stiglec, Frederic Veyrunes,
and Jennifer A. Marshall Graves

The peculiar cytology and unique evolution of sex chromosomes raise many fundamental questions. Why and how sex chromosomes evolved has been debated over a century since H.J. Muller suggested that sex chromosome pairs evolved ultimately from a pair of autosomes. This theory was adapted to explain variations in the snake ZW chromosome pair and later the mammal XY. S. Ohno pointed out similarities between the mammal X and the bird/reptile Z chromosomes forty years ago, but his speculation that they had a common evolutionary origin, or at least evolved from similar regions of the genome, has been undermined by comparative gene mapping, and it is accepted that mammal XY and reptile ZW systems evolved independently from a common ancestor. Here we review evidence for the alternative theory, that ZW ⇔ XY transitions occurred during evolution, citing examples from fish and amphibians, and probably reptiles. We discuss new work from comparative genomics and cytogenetics that leads to a reconsideration of Ohno's idea and advance a new hypothesis that the mammal XY system may have arisen directly from an ancient reptile ZW system.

Introduction

Nearly all vertebrates have separate males and females, but sex is determined in different ways. Many fish and reptile species use an environmental cue, commonly temperature, to trigger male-determination (environmental sex determination or temperature sex determination). In other fish and reptile species, as well as in all amphibians, birds, snakes and mammals, sex is genetically determined (genetic sex determination). Amongst species using genetic sex determination, some are male heterogametic — that is, the male is heterozygous at a sex determining locus — while others are female heterogametic. Many such species have reinforced this allelic difference by differentiating sex chromosomes, some to a remarkable extent. Male heterogametic species have XX females and XY males, whereas female heterogametic species have ZW females and ZZ males (Figure 1).

The best studied vertebrate sex chromosomes are the XY system of mammals, and the ZW systems of birds and snakes. These systems show superficial similarity in that the X and Z are large chromosomes bearing many genes that seem to have little to do

Research School of Biological Sciences, The Australian National University, Canberra, ACT 2601, Australia.
Email: Tariq.Ezaz@anu.edu.au

with sex determination, whereas the Y- and W-chromosomes are smaller, highly heterochromatic and retain few active genes. The mammalian Y-chromosome specializes in male sex and spermatogenesis.

Early speculations on the origin of the *Drosophila melanogaster* X- and Y-chromosomes proposed that sex chromosomes started out as an ordinary autosomal pair. Then, one member acquired a sex determining allele and accumulated male-advantage genes in a non-recombining region that progressively degraded [1]. The same process is proposed to account for the large X and small, gene-poor Y in mammals [2,3]. What causes Y or W degradation has been debated over decades. Two main mechanisms have been identified in mammalian Y-chromosome degradation: a higher mutation rate and inefficiency of selection on a non-recombining chromosome [3,4].

The opposite process, i.e. accumulation of female-advantage genes and degradation of the W-chromosome, was predicted for ZW species. Ohno [5] observed that the Z- and W-chromosomes of different snake families appeared to represent intermediates in such a W-degradation process. Bird sex chromosomes, too, offer a range of degree of differentiation, and there is now molecular evidence for degradation of the W-chromosome in avian lineages [6,7]. Ohno [5] also observed that the Z-chromosome appeared to be similar in all snakes, as did the Z in all birds; in fact, there were similarities that suggested a highly conserved ZW-system in birds and reptiles. He went further to speculate, based largely on the absolute size and similarities of some sex-linked phenotypes, that the bird Z and mammal X shared genes and evolved via parallel pathways or even descended from a common ancestor.

Forty years later, Ohno's bold theory is all but forgotten. Gene mapping and chromosome painting confirm his hypotheses that the mammalian X is highly conserved — at least in placental mammals [8] — and that the avian Z is also highly conserved [9]. However, gene mapping has decisively shown that the avian Z-chromosome has no homology to the human X [10]. Therefore, the accepted theory is that the bird ZW- and the mammalian XY-systems originated independently from two different autosomal regions in a common ancestor, which probably had temperature-dependent sex determination [11]. Moreover, gene mapping shows that the Z of at least one snake species is not homologous to the bird Z-chromosome [12].

However, new molecular cytogenetic and comparative gene mapping methods greatly expand our view of how far and how fast sex chromosome systems can change over time. We now have complete genome sequences for three fish, a toad, a bird, and many mammals from all three major groups. We have dense gene maps of many diverse species and cytological methods for detecting sexually heteromorphic regions and identifying molecular homology between species.

Figure 1. Vertebrate sex determination systems.

Phylogeny of major vertebrate clades showing the sex determining systems found in members of the respective clade. 'Mulitple' indicates involvement of more than one pair of chromosomes in sex determination. Dates of divergence are taken from [42,82,83]. TSD: temperature-dependent sex determination.
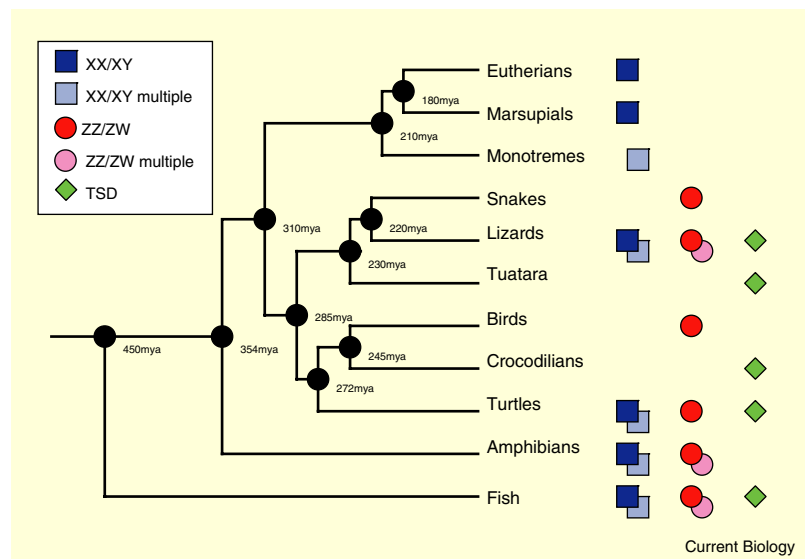
Some observations directly challenge the accepted view that sex chromosomes have evolved independently in different vertebrate groups.

There is a fundamental difference between male and female heterogametic systems; yet, transitions between ZW and XY systems have happened many times during the course of evolution in fish, amphibians and reptiles, although we do not understand how. It has often been assumed that transition requires a spell of temperature-induced sex determination as an intermediate stage, but there are also direct mechanisms for transition. It is generally considered that homomorphic sex chromosomes are a necessary prerequisite for transition between sex-determining systems. Highly differentiated sex chromosomes are likely to pose a barrier to the subsequent transition, as WW- or YY-genotypes are likely to be lethal, if the W or Y is highly degraded [13]. Heteromorphic sex chromosome systems are thought to evolve from an initial state of sex chromosome homomorphy through increasing — and presumably irreversible — stages of differentiation, until a point of cytologically distinguishable sex chromosome heteromorphy is attained [1,2,13]. Here we examine some of the evidence for the relationship and transition between ZW and XY sex chromosomes in vertebrates from fish to mammals. We reconsider Ohno's hypothesis that vertebrate sex chromosomes were fashioned from the same ancestral material and that transition between these two heterogametic systems happened frequently during the course of evolution.

Sex Chromosomes and ZW ⇔ XY Transitions in Fish

One of the most interesting vertebrate groups in which to examine sex chromosome evolution are fish. This large vertebrate group displays the widest variety of sex determination and sex chromosomal systems, including gonochorism (separate sexes), hermaphroditism (individuals displaying both sexes) and unisexuality (all female-species). In gonochoristic species, the two sexes may be determined genetically — ranging from determination by a single allele to chromosomal

sex determination. There is also polygenic sex determination and sex determination by genotype-environment interaction. Temperature sex determination has been unequivocally established in at least one species, the silverside, *Menidia menidia* [14].

Morphologically differentiated sex chromosomes have been identified only in about 10% (176 species out of 1700 species) of fish studied karyologically [15]. These include ZW and XY systems with eight different variants [16], among which male heterogamety is twice as common as female heterogamety. Within XY or ZW systems, variation in sex chromosome morphology is readily explained by addition/deletion of heterochromatin [17], or chromosomal fission or rearrangement by Robertsonian or tandem fusion to produce a multiple system. More interestingly, some completely novel sex determining systems have evolved by gene duplication or retrotransposition, or by variation and deletion of sequences. Did fish ZW and XY systems evolve independently from an ancestral system, or have there been recent ZW⇔XY transitions? It was proposed that single gene sex determination is ancestral to both male and female heterogamety in teleost fish [5,18]. XY and ZW systems could, therefore, have evolved independently following fixation of dominant male or female sex determiners, rather than requiring transition from one to another system.

A few fish sex chromosome systems are very old and conserved, as might be expected from independent evolution of male and female heterogamety. For instance, a ZW sex chromosomal system has been conserved in several species of the fish families Aulopidae and Synodontidae for at least 60 million years [19,20]. Similar modes of sex chromosomal systems are often conserved within the species in the same genus or family [15,21]. However, many fish sex determining systems appear to have evolved recently, judging by the minimal sequence difference between the sex chromosomes, and observations that sequences that are sex specific in one species are autosomal in sister species (e.g. in sticklebacks [22,23] and tilapia [24]). Most spectacularly, a retroposed copy of the

DMRT1 gene took over a male determining function and initiated differentiation of a new Y chromosome in medaka, but not in closely related species [25].

The patchy distribution of different sex chromosome systems in fish suggests that numerous transitions between different ancestral modes (e.g. environmental, unisexuality, hermaphroditism, ZW, XY) have occurred during the course of evolution [21]. For instance, teleost fish display a haphazard distribution of ZW and XY sex chromosomal systems [21,26,27], suggesting that ZW- and XY-systems evolved repeatedly in this ~235 million year old lineage, and transitions were frequent. It might be expected that such transitions were preceded by sex chromosome polymorphism between populations. However, little intra-species variation and only one case of intra-population sex chromosome variation have been described in fish [28,29]. The best-studied example is the platyfish (*Xiphophorus maculatus*), which has a well-studied homomorphic sex chromosome complex of X-, Y-, W- and Z-chromosomes within the same populations [29]. Different combinations of these sex chromosomes give rise to different phenotypic sexes, and it has been proposed that sex is determined by a dosage of sex determining and regulatory genes distributed in different types of sex chromosomes and autosomes [29]. A modified XY system with autosomal influence has been described in two other platyfish species (*X. nezahualcoyotl* and *X. milleri*) [28], while a strict XY-system is found in *X. cortezi* and a ZW-system in *X. alvarezi* [28–30]. The platyfish may, therefore, be caught in a transition between XY- and ZW-systems. Taken together, fish provide examples for rapid transitions between a ZW- and a XY-system and at least one example for an interaction of both systems.

ZW⇔XY Transitions in Amphibia

Amphibian sex determination is of special interest, because — although all amphibians investigated display genetic sex determination — both male and female heterogamety are represented, often in the same family, genus, species or even population. There are no reports of amphibians with environmental or polygenic sex determination in the wild, although some species show spontaneous sex reversal, and others show a temperature effect in the laboratory [31,32].

Only 4% of the amphibians karyotyped have cytologically differentiated sex chromosomes [33–35], most of which are ZW. One species has multiple sex chromosome systems [36], and an endemic New Zealand frog, *Leiopelma hochstetteri,* has OO males and OW females [37]. In this species, the Z chromosomes are absent from both males and females and the W chromosome is present only in females (i.e., the diploid chromosome complement of males is 2n = 22, while that of females is 2n = 22 + W). There is some consistency in which chromosome acts as the sex pair, with chromosomes 4, 7, 8, 11 or 13 taking this role in many species [35,38]. Morphological differences between sex chromosomes of different amphibians are due to relatively trivial changes, including heterochromatin accumulation, pericentric inversions, insertions or deletions and the presence of supernumerary chromosomes. Phylogenetic analysis of amphibian sex chromosomal systems suggests that a ZW-system is ancestral, but XY-systems evolved several times within most lineages [33].

Amphibians provide a unique example of population variation for male and female heterogamety. In the Japanese frog *Rana rugosa*, XY- and ZW-systems evolved within different populations in a relatively short period of time [39,40]. Some populations show female heterogamety and others male heterogamety. Homology of the XY and ZW pairs implies that they evolved from the same chromosome pair. There are also two intermediate conditions with male heterogamety but without sex chromosomal heteromorphy. WW-individuals can be produced by induced gynogenesis in two populations of *R. rugosa*. These WW-individuals are lethal and die after hatching [41]; however, nothing has been reported about the fitness of YY-individuals. Evolution of ZW and XY chromosome systems in *R. rugosa* is proposed to be the result of hybridisation of two ancestral forms with male heterogamety but homomorphic sex chromosomes. Different chromosome rearrangements (primarily pericentric inversions) occurred in two hybrid populations. In one of these populations, the sex chromosome pair retained a male-determining locus (XY male heterogametic), and in another the sex pair acquired a dominant female determining gene, evolving into a ZW female heterogametic system. Thus amphibians appear to have undergone independent ZW⇔XY transitions, and present a clear case of ZW/XY polymorphism.

Sex Chromosomes in Reptiles and Birds

Fossil and molecular evidence shows that reptiles and birds shared a common ancestor ~285 million years ago [42], and in the phylogenetic tree birds nest with reptile groups (that is, birds are a group of reptiles). They show similarities to reptiles in chromosome number and morphology as well as the division into relatively few large macrochromosomes and many tiny microchromosomes. Reptiles show both genetic and temperature-dependent sex determination. All crocodilians, most turtles and many lizards display temperature-dependent sex determination [43,44]. Reptiles with genetically determined sex are either female or male heterogametic, but few groups have cytologically detectable sex chromosomes. Well-differentiated Z and W chromosomes are ubiquitous only in birds and snakes.

All the hundreds of bird species that were tested display ZW female heterogamety. The Z chromosome is very uniform in size, either the fourth or fifth largest, but the W chromosome varies from virtual homomorphy in the flightless ratites to extreme differentiation in most carinates (Figure 2). Chromosome painting using DNA from a flow-sorted chicken Z chromosome shows that the Z is genetically homologous across birds [9,45–51], and gene mapping demonstrates that the W is a relic of the Z that has been degraded to different extents in different bird lineages [52] (Figure 2). In the absence of sex chromosome aneuploids — which are evidently lethal [53] — it is not known whether the W contains female determinants, but extensive search failed to find early expressed W-specific genes [54]. Alternatively or in parallel, there may be Z

Figure 2. Sex chromosomal morphology in groups of snakes and birds.

The most frequent morphologies are shown. Only those avian sex chromosomal genes are shown here, which have been localised onto carinate, ratite and snake sex chromosomes. However, several other chicken sex chromosomal genes have been mapped onto the short arm of Japanese rat snake chromosome 2 (chromosome 2p) [12], suggesting a homology between chicken Z and rat snake autosome 2; this may also indicate many chromosome translocations in rat snake involving sex chromosomes and autosomes. REP: repetitive sequences.
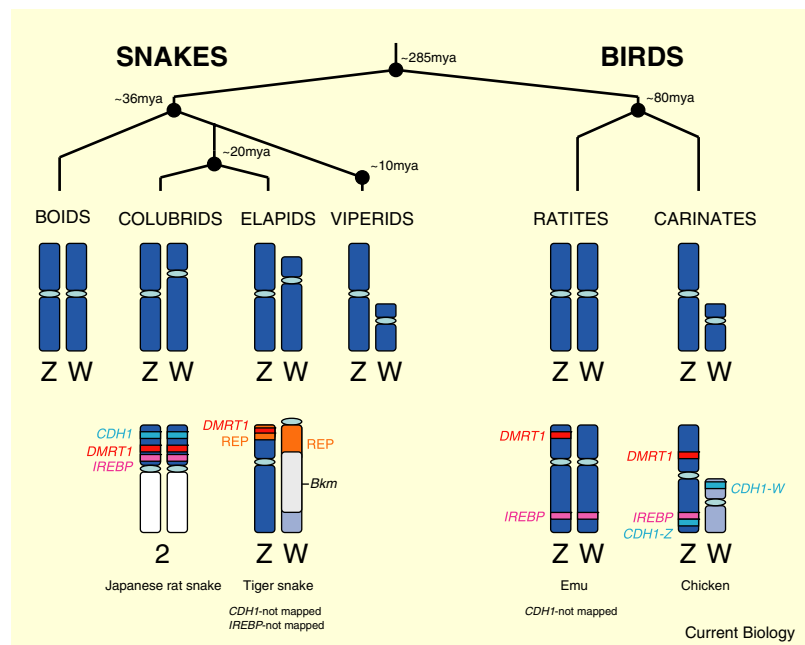
dosage-dependent male determinants. A credible candidate sex-determining gene is DMRT1, mutations in which cause sex reversal in humans. In all birds investigated so far, DMRT1 lies on the Z chromosome [55–57], however, other genes from the Z-specific region cannot be excluded as candidates. The best evidence that DMRT1 is the primary avian sex-determining gene is that in emus (*Dromaius novaehollandiae*) — a ratite with homomorphic sex chromosomes — DMRT1 is located on the Z, but not on the nearly homologous W chromosome [57]. This is consistent with the hypothesis that DMRT1 may play an important Z-dosage role in all birds. The gene-content of the chicken Z chromosome appears to be biased, as is the content of the human X. Using the chicken genome database, we find that the chicken Z chromosome — like the human X [58] — is significantly depleted in cancer genes (R. Stiglec, unpublished observation). This condition appears to be universal for heteromorphic sex chromosomes and protects the hemizygotic sex from recessive cancer-causing mutations of sex chromosome-linked cancer genes.

As mentioned above, snakes all have a ZW-system with a Z of similar size and morphology to that of birds. As for birds, snake W chromosomes show cytological gradation from virtual homomorphy in boid snakes (pythons and their relatives) to extreme differentiation in vipers. These observations were the original inspiration for Ohno's hypothesis that the W chromosome has been degraded to different extents in different snake lineages. Although no genes have yet been mapped to a snake W, it is expected that — as for the degradation of mammalian Y — the W represents a more or less degraded version of the Z (Figure 2). Close examination of snake chromosomes suggested that accumulation of heterochromatin is an early change that initiated sex chromosome differentiation, at least in some snake groups [59–61].

Based on the similarity in size of the Z chromosome across many snake families, Ohno [5] suggested that the Z chromosome is conserved across all snake species. However, it is difficult to test this hypothesis with the limited and contradictory gene mapping data for snakes. Recently, we mapped DMRT1 to the sex chromosomes in the tiger snake, *Notechis scutatus*, an Australian elapid snake (R. Stiglec, unpublished observation). However, many genes including DMRT1 were recently physically mapped in a colubrid, the Japanese rat snake *Elaphe quadrivirgata* [12]. Surprisingly, DMRT1 and other genes from the chicken Z chromosome mapped onto chromosome 2 in the rat snake, whereas two autosomal chicken genes mapped onto the rat snake Z chromosome. This could mean that the sex chromosomes are genetically different in the two snake families, completely undermining the assertions of Ohno's hypothesis. Alternatively, the large rat snake Z could be a compound chromosome derived from reciprocal translocation of the original Z with an autosome. Several examples for translocations between sex chromosomes and autosomes have been proposed. For instance, the ancestral Y chromosome of the genus *Drosophila* was translocated to an autosome in *Drosophila pseudoobscura* and a completely new region of the genome took over function as a neo-Y [62]. Comparative mapping of Z-linked genes in other snake groups is required to test further the hypothesis that the snake ZW system is ancient.

Similarities in Z chromosome size and morphology, as well as some sex-linked phenotypes, led Ohno [5] to suggest that the snake and bird Z — and therefore W — chromosomes were genetically similar. Did birds retain an ancestral reptilian ZW pair? Or did they evolve in parallel from the same ancestral chromosomal pair? There are no cross-species chromosome painting data with which to assess homology of snake Z or W chromosomes, and gene mapping data are

contradictory. Comparative chromosome painting and gene mapping between snakes and birds could test the hypothesis that the Z is genetically homologous between these two groups.

Sex chromosomes in other reptiles are much more variable than those of birds and snakes. Many lizards and a few turtles have heteromorphic sex chromosomes detectable by traditional cytological techniques [63,64]. Lizards — the largest reptile group — have the most diverse array of sex chromosome systems among the reptiles. Sex chromosomes have been found in 172 of the 953 lizard species karyotyped (of the total 4765 extant lizard species), representing 10 of the 18 lizard families. Male and female heterogamety, as well as multiple chromosomal sex determining systems have been described in several families [63–65]. In a few lizards of the families Iguanidae and Lacertidae, sex is evidently determined by microchromosomes, [66,67], suggested to have been recently derived by macrochromosome degradation and deletion [67]. However, comparative genome hybridisation and C-banding in several species of dragon lizards revealed completely differentiated W and Z chromosomes [68] (T. Ezaz, unpublished observation), suggesting an ancient and stable sex chromosome system like those of snakes and birds. C-banding variation among different species and populations suggests different degrees of degradation in these lizard groups.

Most turtles have a temperature-dependent sex determination system, suggesting that it is ancestral to this group. Differentiated sex chromosomes occur in only four of the 154 turtle species karyotyped (of a total of 254 turtle species); three of these have XY-systems and one has a ZW-system [65]. However, X and Y microchromosomes were recently detected by high resolution molecular cytology techniques in a chelid turtle *Chelodina longicollis* [69] and might be more common than previously supposed. The minimal difference between *C. longicollis* X and Y chromosomes suggests an early stage of differentiation, implying a recent transition from temperature-dependent to genetic sex determination [69].

In summary, reptiles show many different sex determining and sex chromosome systems. Among vertebrates they provide the best evidence for degradation of the Y or W chromosomes. Within the reptiles, there are many examples of transitions between temperature and genetic sex determination systems, but no clear cases of ZW⇔XY transitions.

Mammalian Sex Chromosomes and the Hypothesis of a ZW⇔XY Transition

The best known vertebrate sex chromosomes are our own human X and Y, closely followed by those of mouse. The human X is a medium sized chromosome (∼155 Mb) containing about 1400 genes. The X of other placental mammals has a similar size and gene content and even gene order [8], except for the mouse X, which is highly rearranged. Although the X contains a mixture of housekeeping and specialized genes, there is a strong bias towards genes involved in sex and reproduction, as well as brain function [70]. The most plausible explanation of this biased gene content is that alleles of X-borne genes that confer an advantage to males accumulate on the X because they are immediately exposed to selection in males but not females, a hypothesis advanced several decades ago [71]. There is also a strong bias against cancer genes on the human X [58], which presumably mitigates the lethal effect of somatic mutations in X-borne recessive tumour suppressor genes.

The human Y is much smaller than the X, containing only 45 active genes coding for unique proteins and only 27 of those in the male-specific region; however, there are several multicopy genes and there are many transcribed pseudogenes. The Y chromosome is unique in its specialization for sex and reproduction, the result of selection for male advantage genes, especially those with deleterious effects on females. One of these specialized genes is SRY, which triggers testis determination in XY embryos [72].

The proposal that the mammalian X and Y evolved from an ordinary autosome pair [3,4] accounts for the homology between the X and Y within the small terminal shared ("pseudoautosomal") regions, and homology between pairs of genes on the X and Y. Much evidence from many systems supports the hypothesis that the mammalian Y chromosome has degraded over the last 300 million years at a rate that would see it disappear in 15 million years or so. Many gene copies on the Y chromosome have evolved male-specific functions, including SRY, which evolved from a widely expressed gene with functions in brain development.

Comparisons between distantly related mammal groups showed that the X and Y of humans and other placental mammals both consist of an ancient region that was differentiated more than 200 million years ago, and at least one more recently added region that is still not entirely differentiated [73,74]. Comparative gene mapping between humans and chicken shows that this ancient region is represented in chickens by chromosome 4p and microchromosomes that are separate in fish ancestors. A region that was recently added to this ancient X in placental mammals is homologous to part of chicken chromosome 1 [75,76]. The mammal-specific gene SRY is represented by a conserved gene SOX3 on chicken chromosome 4p. Conversely, the chicken ZW-pair is represented in humans by most of chromosome 9, including the putative bird sex determining gene DMRT1, plus small regions of chromosome 5. Thus, there appears to be no homology at all between the mammalian XY- and the bird ZW-pairs, suggesting that the mammalian XY-pair and the bird ZW-pair evolved independently from two different autosomes [11]. Recent reconstructions of an ancestral fish karyotype [77] reinforce the idea that the ancestral blocks that gave rise to these two sex chromosome systems were separate.

However, two recent observations challenge the theory that higher vertebrate sex determining systems evolved independently. The platypus, an egg-laying monotreme mammal, possesses five X chromosomes and five Y chromosomes in the male [78]. These pair up in an $X_1Y_1X_2Y_2X_3Y_3X_4Y_4X_5Y_5$ translocation chain at male meiosis and segregate to form female ($X_1X_2X_3X_4X_5$), and male ($Y_1Y_2Y_3Y_4Y_5$) gametes [79].

Gene mapping shows that the X_1 at one end of the male meiotic translocation chain has homology to the human X, and the X_5 at the other end has homology to the bird ZW. The platypus sex-determining gene is not known; SRY is absent, and DMRT1 is on an X chromosome and, therefore, has a male:female dosage of 1:2, the reverse of the 2:1 dosage in birds.

Another challenge to the hypothesis is provided by studies of the biased gene content of sex chromosomes. The human X chromosome is depleted of cancer genes, as might be expected because otherwise hemizygous males would be very susceptible to cancer caused by a single somatic mutation [80]. Our finding that the chicken Z chromosome, too, is significantly depleted in cancer genes (R. Stiglec, unpublished observation) was, therefore, not unexpected, and suggests that a bias against such genes is a general property of a chromosome that is present in only one copy in half the population. However, our discovery that the human equivalents of the chicken Z, i.e. regions of human chromosomes 9 and 5, are similarly biased was initially puzzling, especially as regions of human chromosomes 5 and 9 homologous to chicken autosomes (chicken chromosomes 2, 13, 17) are not depleted in cancer genes. Our interpretation is that mammal ancestors shared the ZW chromosome pair with birds and reptiles, and that the depletion of cancer genes on the Z was retained on the orthologous autosomal regions after the transition to a different sex pair. Though indirect, this recent evidence is consistent with the possibility that the SRY-driven mammalian XY system may have taken over from an ancient reptilian ZW system, some time around the divergence of therian mammals (placentals and marsupials) from monotremes 210 million years ago.

How did the sex determining genes change during this transition? In therian mammals, the Y-borne SRY acts as a dominant testis-determining gene. This gene is absent in reptiles and birds, and also could thus far not be found in the platypus, although the X-borne gene SOX3 from which it evolved is highly conserved and is located in the conserved region of the therian X. Although it is still uncertain which genes are sex-determining in birds and reptiles, DMRT1 seems to be the most promising candidate. It is, therefore, possible that in the mammalian lineage the new male dominant gene SRY evolved from SOX3 on one member of an autosomal pair translocated to the Z, the 'proto-Y', in a ZZ male and that this gene took over the control of the sex-determining pathway. As SRY acts upstream of DMRT1 in the sex determination pathway [81], its male-dominant action is likely to have been epistatic to DMRT1. Thus DMRT1 expression was brought under the ultimate control of SRY to produce XYZZ males and XXZZ females. This must have occurred when sex chromosomes were minimally differentiated, as such transitions are unlikely to be fixed in a system with highly heteromorphic sex chromosomes, in which YY or WW individuals are lethal. Comparative gene mapping and chromosome painting involving various taxa is required to clarify further how transitions can occur between different heteromorphic sex chromosomes.

Conclusions

Male and female heterogamety resulting from XY- and ZW-systems have been thought to evolve independently from a common ancestor with environmental sex determination, or to go through an intermediate stage in which sex is determined by environmental cues. However, there are at least some examples which support the alternative theory that direct ZW ⇔ XY transitions have occurred — at least in fish and amphibians, and probably in reptiles. The extraordinary sex chromosome complex in the platypus and the gene bias in mammalian orthologues of the chicken Z provide some support for the hypothesis that such a transition may also have occurred in the early history of mammals. The alternative hypothesis of vertebrate sex chromosome evolution is that the mammalian XY-system may have arisen directly from an ancient reptilian ZW-system, rather than an intermediate or transitory state of environmental sex determination. This hypothesis will be testable with data from the emerging platypus genome sequence and by comparative mapping studies with birds, reptiles and amphibians, which will give new insights into the sex chromosome evolution of vertebrates.

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