

A new look at the evolution of avian sex chromosomes

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Abstract. Birds have a ubiquitous, female heterogametic, ZW sex chromosome system. The current model suggests that the Z chromosome and its degraded partner, the W chromosome, evolved from an ancestral pair of autosomes independently from the mammalian XY male heteromorphic sex chromosomes – which are similar in size, but not gene content (Graves, 1995; Fridolfsson et al., 1998). Furthermore the degradation of the W has been proposed to be progressive, with the basal clade of birds (the ratites) pos-

sessing virtually homomorphic sex chromosomes and the more recently derived birds (the carinates) possessing highly heteromorphic sex chromosomes (Ohno, 1967; Solari, 1993). Recent findings have suggested an alternative to independent evolution of bird and mammal chromosomes, in which an XY system took over directly from an ancestral ZW system. Here we examine recent research into avian sex chromosomes and offer alternative suggestions as to their evolution.

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The chicken Z and W chromosomes

The Class Aves has a consistent karyotype ($2n = \sim 80$) (Oguma, 1938; Yamashina, 1944; Ohno et al., 1964; Takagi and Sasaki, 1974), with the Z chromosome always a similar size (7–10% of the genome; Ohno et al., 1964; Takagi et al., 1972; Schmid et al., 2005) and, depending on the species, representing either the fourth or fifth largest chromosome pair (Suzuki, 1930; Ohno et al., 1964). The W chromosome, however, is very different in size in different bird families, being virtually the same size as the Z in ratites, but very small and heterochromatic in most carinates (Ohno et al., 1964; Takagi and Sasaki, 1974) (Fig. 1).

The chicken (*Gallus gallus*) is the foremost model avian organism, and has the only fully sequenced avian genome (Hillier et al., 2004). The chicken has a diploid number of 78 (Masabanda et al., 2004). Cytologically, the chicken Z chromosome is the fifth largest macrochromosome (Suzuki, 1930). Both the Z chromosome (GGAZ) and W chromosome (GGAW) are submetacentric, with GGAZ (76.4 Mb) being significantly larger than GGAW (0.026 Mb) (Karolchik et al., 2003; NCBI chicken build 2.1, November 2006).

According to the latest chicken genome assembly draft, GGAZ contains a total of 841 protein coding genes, while in contrast, GGAW contains only 40 protein coding genes (Karolchik et al., 2003; NCBI chicken build 2.1, November 2006). Even this small number of W genes is questionable as there are many database assembly problems with the GGAW sequence, especially within the original draft assembly. We have recently mapped 17 of these 'W' genes to GGAZ (Stiglec et al., 2007). To date only a handful of genes, including *HINTW*, *ATP5A1* and *CHD1*, have been physically mapped to GGAW (Fridolfsson et al., 1998; O'Neill et al., 2000; Backstrom et al., 2005); all but three (*ETM1/FET1*, *2d-2D9*, *2d-2F9*) have homologues on GGAZ (Reed and Sinclair, 2002; Yamada et al., 2004).

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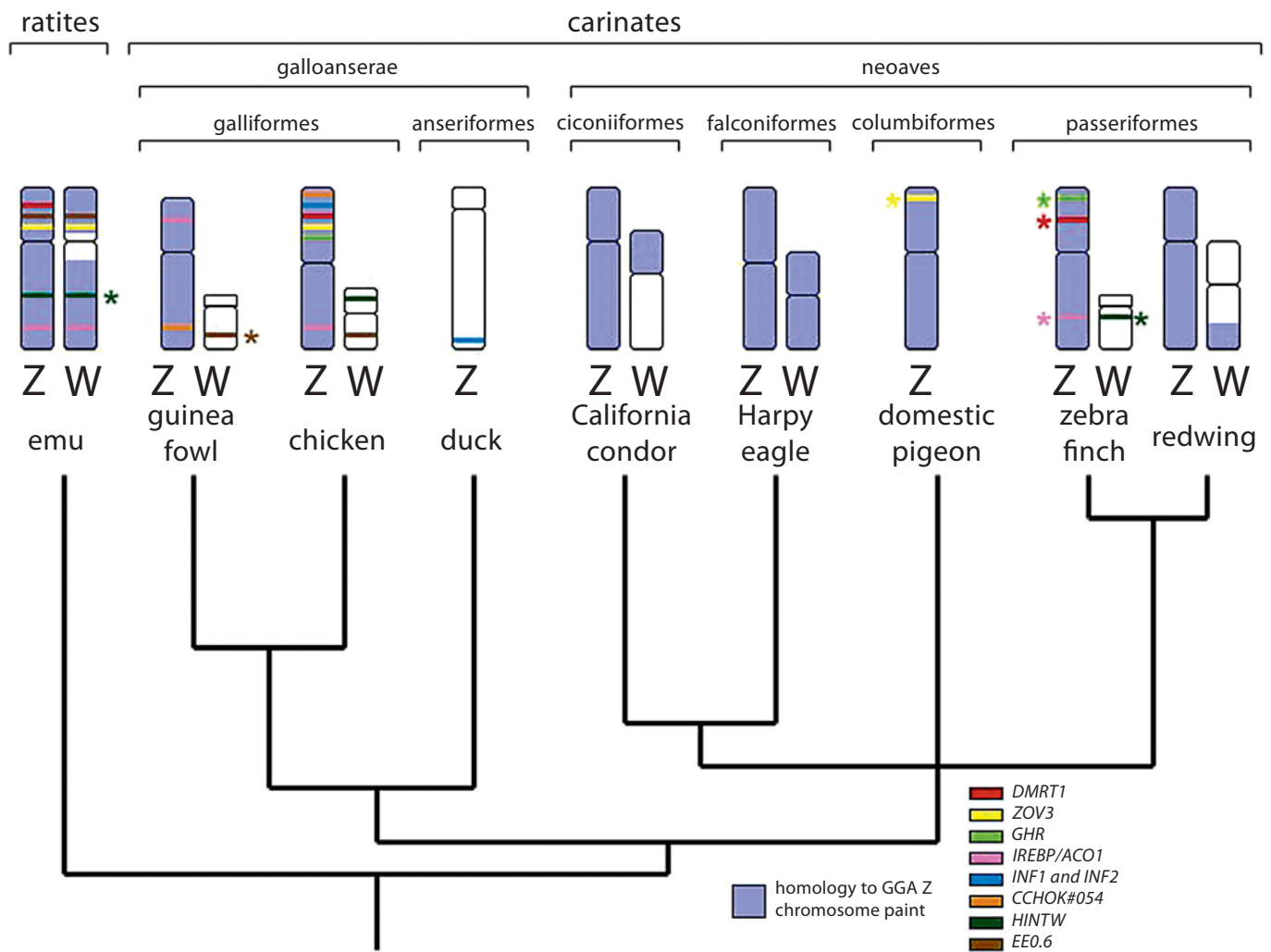


Fig. 1. GGZ chromosome painting and sex-linked comparative gene mapping across several bird species. No cross-species Z chromosome painting has been conducted on the duck, thus Z homology can only be determined by comparative gene mapping (e.g. *INF1* and *INF2*). The lack of W chromosomes for duck and domestic pigeon is a result of insufficient cross-species painting data for these chromosomes and does not represent their karyotype. * Indicates genes that

have been localised to a sex chromosome by non-cytological means (such as Southern blotting), but whose specific loci are currently unknown. Adapted from Ogawa et al. (1997, 1998), Nanda et al. (1998), Shetty et al. (1999), Nanda et al. (2000), Schmid et al. (2000), Raudsepp et al. (2002), Shetty et al. (2002), Shibusawa et al. (2002, 2004a, b), Kasai et al. (2003), Derjusheva et al. (2004), de Oliveria et al. (2005), Itoh and Arnold (2005) and Itoh et al. (2006).

In XX female mammals, X chromosome dosage compensation is achieved via the inactivation of one of the X chromosomes. There is no equivalent Z-inactivation in ZZ male chickens, as biallelic expression of Z-linked genes has been observed for two genes (Kuroiwa et al., 2002). However, comparisons of expression levels of nine Z-borne genes between males and females showed that most genes were equally expressed between the sexes. At least three genes escape dosage compensation (McQueen et al., 2001; Kuroiwa et al., 2002). Mechanisms other than Z-inactivation have been proposed, including the up-regulation of Z-linked genes in females and/or the down-regulation in males; or, translational compensation of Z-linked mRNAs in one or both of the sexes (Ellegren, 2002). It has been suggested that a W-linked locus might control the up-regulation of Z-

linked genes in ZW females (Graves, 2003). This hypothesis would also explain why sex chromosome aneuploidy is not observed in birds – ZZW birds with twice the amount of Z-linked gene expression, and ZO birds with only half the amount would probably be unviable (Graves, 2003).

Avian sex determination

It is unclear whether avian sex determination is due to a W-linked, ovary-determining factor or to a Z-linked, testis-determining factor that escapes dosage compensation, or an interaction of W- and Z-borne factors. There are strong candidates for these roles: *HINTW* on the W and *DMRT1* on the Z.

HINTW (also known as *ASW* or *PCKI-W*) is one of the strongest candidates for an ovary-determining factor in birds. It is W-specific in 40 species from several different carinate families (O'Neill et al., 2000; Hori et al., 2000) and shows female-specific expression in developing genital ridges (O'Neill et al., 2000). Although in carinates the Z-linked homologue of *HINTW* shows little sequence or copy number similarity (with the Z-linked homologue usually present in a single copy, whilst *HINTW* is present in multiple copies) the Z and W homologues in ratites appear to be identical, insofar as they have a similarly low copy number (Hori et al., 2000), making it unlikely to be the primary sex-determining gene in birds. As neither ratite *HINTW* nor its Z-linked homologue have been sequenced, we can not be sure of the exact level of similarity between the two and whether they are more closely related to the carinate *HINT-W* or *HINT-Z*. Other likely W-linked candidate genes are *ETM1*, *2d-2D9* and *2d-2F9* which do not have homologues on GGAZ (Reed and Sinclair, 2002; Yamada et al., 2004).

DMRT1 is the only gene involved in sexual differentiation found in all metazoans, playing an important male-determining role in mammals, birds, amphibians, reptiles, insects and nematodes (Raymond et al., 1999; Kettlewell et al., 2000; Nanda et al., 2000; Shibata et al., 2002). In eutherian mammals, *DMRT1* is autosomal, yet two copies are required for testis determination (Raymond et al., 1999, 2000).

DMRT1 is located on the Z chromosome in six species from three representative avian families (galliformes, passerines and ratites) (Nanda et al., 2000; Shetty et al., 2002; Itoh et al., 2006) (Fig. 1). Unlike several other chicken Z-linked genes, *DMRT1* shows no dosage compensation in males and has higher expression in males than females before and during gonadogenesis (Raymond et al., 1999; Smith et al., 1999; Oreal et al., 2002), suggesting an important dosage sensitive role in male determination and differentiation. Perhaps 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 the nearly homologous W (Shetty et al., 2002). This is compatible with the hypothesis that *DMRT1*, or a gene close to it, plays an important Z-dosage role in all birds.

Sex chromosomes in different avian species

The sex chromosomes of different bird species have been compared by cytological methods, particularly comparative chromosome painting and gene mapping. Cross-species chromosome painting and comparative mapping of sex chromosome-linked sequences has demonstrated Z chromosome conservation throughout the Class Aves.

Chromosome painting refers to hybridization of DNA from isolated chromosomes of one species onto the chromosomes of another. As the chicken is the best-studied bird genome, most comparative studies use chicken chromosome paints and genes.

The first of the chromosome painting studies was the most dramatic since it compared the chromosomes of the chicken with the distantly related emu (Shetty et al., 1999). Cross-species chromosome painting of emu chromosomes with chicken chromosome paints demonstrated strong homology between all nine macrochromosomes of the two species. The only difference was that the chicken chromosome 4 (GGA4) paint also hybridises to a pair of microchromosomes: it turns out that the short arm of GGA4 (orthologous to human Xq) is equivalent to a microchromosome in all birds other than the chicken. The GGAZ paint completely hybridises to the emu Z, showing that the two chromosomes are homologous. Painting with the chicken Z also produced signal all along the homomorphic emu W, except for a small region on the short arm that corresponds with the *DMRT1* locus, as well as a centromeric region. This demonstrates that the emu Z and W chromosomes are nearly homologous (Shetty et al., 2002), and suggests that they undergo Z-W recombination at meiosis except for these two regions that might contain a sex-determining gene, e.g. *DMRT1* (Fig. 1).

These chicken/emu painting results have since been confirmed by comparative gene mapping. The orthologues of several GGAZ sequences have been mapped to the emu and ostrich (*Struthio camelus*) sex chromosomes. The chicken Z-linked genes *IREPB* and *ZOV3* along with the W-linked EE0.6 marker all localise to both the emu and ostrich Z and W chromosomes (Ogawa et al., 1998), further demonstrating the homology between these two homomorphic sex chromosomes. *DMRT1*, on the other hand, has been shown to localise to the emu Z chromosome, on the distal region of the short arm, but not on the emu W (Shetty et al., 2002) (Fig. 1).

Among the carinate birds, strong sex chromosome dimorphism is observed between the Z and W chromosomes. Intra-species comparisons show that the Z chromosome is similar in size throughout all lineages, although centromere positions vary between species (Takagi and Sasaki, 1974). In contrast, the W chromosomes show major differences in size between species. In some rare cases, like the eagle owl (*Bubo bubo*), there is Z chromosome heteromorphism due to differing amounts of constitutive heterochromatin (Guttenbach et al., 2003).

Cross-species chromosome painting, using the GGAZ paint as a probe, has been conducted on several galliformes (Schmid et al., 2000; Shibusawa et al., 2002, 2004a, b; Kasai et al., 2003) three passeriformes (Derjusheva et al., 2004; Itoh and Arnold, 2005), and single species of columbiformes (Derjusheva et al., 2004), ciconiiformes (Raudsepp et al., 2002) and falconiformes (de Oliveira et al., 2005). In all cases, the GGAZ paint entirely hybridised the Z chromosome (Fig. 1), demonstrating a remarkable conservation of Z chromosome homology throughout the avian lineage. In a few species the GGAZ paint also hybridised to parts of the W chromosome (Fig. 1); to the entire W of the Harpy eagle (*Harpia harpyja*), to the entire W short arm in the California condor (*Gymnogyps californianus*), and to the terminal region of the W long arm in the redwing (*Turdus iliacus*)

and chaffinch (*Fringilla colelbs*). In the most comprehensive avian cross-species chromosome painting study to date (Guttenbach et al., 2003) chicken macrochromosome paints were hybridized to the macrochromosomes of nine birds species from six different orders. It is surprising, and disappointing, that the Z chromosome was not analysed during this study.

Comparative gene mapping of chicken Z-linked genes helped to confirm these cross-species Z-paint results (Nanda et al., 2000; Shibusawa et al., 2001, 2002, 2004b), and has also demonstrated Z chromosome homology in species that have not yet undergone cross-species Z painting, such as the anseriformes (Fig. 1) (Nanda et al., 1998; Nanda and Schmid, 2002).

Evolution of the avian Z and W

The remarkable conservation of the avian Z chromosome as demonstrated by cross-species chromosome painting and gene mapping supports Ohno's (1967) hypothesis of a single ancestral avian Z chromosome. Changes in gene order attest to several intra-chromosomal rearrangements (inversions), but an absence of inter-chromosomal rearrangements. Comparative chromosome painting and gene mapping, showing varying degrees of Z-W homology, also support Ohno's (1967) hypothesis that the W chromosome represents a degraded and differentiated Z chromosome.

The most basal bird Order, the ratites, possess Z and W chromosomes that are not only morphologically identical, but genetically homologous, except for a small region around the W centromere, as shown by comparative gene mapping of both GGAZ- and W-linked sequences to both of the sex chromosomes. In contrast, carinate birds generally have strongly differentiated Z and W chromosomes. Although the Z chromosome is highly conserved across all birds, the W chromosome is not – chromosome painting and gene mapping do confirm some homology though. For instance, GGAZ paint hybridises to parts of the W chromosome of many Neoaves species (i.e. redwing, chaffinch, Californian condor and the Harpy eagle). However, no GGAZ paint homology was noted in the W chromosome of any of the Gallanserae, including the chicken itself. GGAW paints have been found not to hybridise across avian species (Raudsepp et al., 2002). This is a situation parallel to the lack of homology of the Y chromosome, even between closely related species (e.g. Toder et al., 2000), which is due to rapidly evolving repetitive sequences (Itoh et al., 2006) on the heterochromatic regions of the W chromosome. However, several chicken W-linked sequences have been localised to the zebra finch W (Agate et al., 2004; Itoh and Arnold, 2005), demonstrating some conservation of the avian W.

Comparative sequence analysis of intron regions of genes with Z- and W-linked homologues in twelve avian species from four representative avian orders (galliformes, anseriformes, passeriformes and strigiformes) allows for the calculation of an approximate timescale for the termination of Z-W recombination; the longer the time since Z-W recom-

ination ceased, the larger the sequence differentiation between the Z- and W-linked homologues. These calculations also reveal the formation of two Z chromosome strata, as recombination continued in one region (stratum 2) after it had stopped in another (stratum 1) (Handley et al., 2004). Thus the avian ZW pair, like the mammal XY pair, differentiated in fits and starts (Graves, 1995, 2006). Stratum 1 appears to have been created by a single inversion on the W chromosome, disrupting its homology to the long arm of the Z, and resulting in the suppression of recombination. This occurred 102–170 MYA, prior to the major avian radiation (Handley et al., 2004). Stratum 2 continued to recombine until after the divergence of extant avian orders. Independent cessation of Zp-W recombination occurred among the different avian lineages between 58 and 85 MYA. The stratum 2 data indicates that Z/W divergence occurred discontinuously, and suggests that fully differentiated avian sex chromosomes evolved independently within different lineages. Fully differentiated avian Z/W sex chromosomes must therefore represent a converged state and not a common ancestral one (Handley et al., 2004). This conclusion appears to be supported by the cross-species Z chromosome painting, which shows GGAZ homology, of varying degrees, to the W chromosome in several carinate species (e.g. Californian condor and the Harpy eagle). This corroborating data, however, might just be superficial, demonstrating only a common GGAZ-linked repeat that is also present in these W chromosomes. Cross-species mapping of W-linked genes in these species is required to resolve this.

Do the avian ZW and the mammal XY share a common ancestry?

The observation that no genes are shared between the avian ZW and the mammal XY led to the wide acceptance of the hypothesis that the two sex chromosome systems evolved independently from different autosomes, probably from an ancestor with no sex chromosomes that determined sex via environmental cues (Graves and Shetty, 2000). However, two recent results lead us to query this assumption.

Monotremes (egg-laying mammals), such as the platypus, are the most basal group of mammals. These extraordinary egg-laying mammals prove to have extraordinary sex chromosomes that appear to link the avian ZZ/ZW and the mammalian XX/XY systems.

Unlike therian mammals (i.e. marsupials and eutherians), which possess an XX/XY male heterogametic sex chromosome system, the platypus has ten sex chromosomes that form a translocation chain at male meiosis. Female platypuses have ten X chromosomes ($X_1X_1X_2X_2X_3X_3X_4X_4X_5X_5$), and males have five X chromosomes and five Y chromosomes ($X_1Y_1X_2Y_2X_3Y_3X_4Y_4X_5Y_5$), which pair in an alternating arrangement at meiosis, and segregate into sperm with either $X_1X_2X_3X_4X_5$ (female producing) or $Y_1Y_2Y_3Y_4Y_5$ (male producing). Extensive gene mapping of human X-linked genes onto the platypus genome has demonstrated homology with the largest X chromosome (X_1) (as

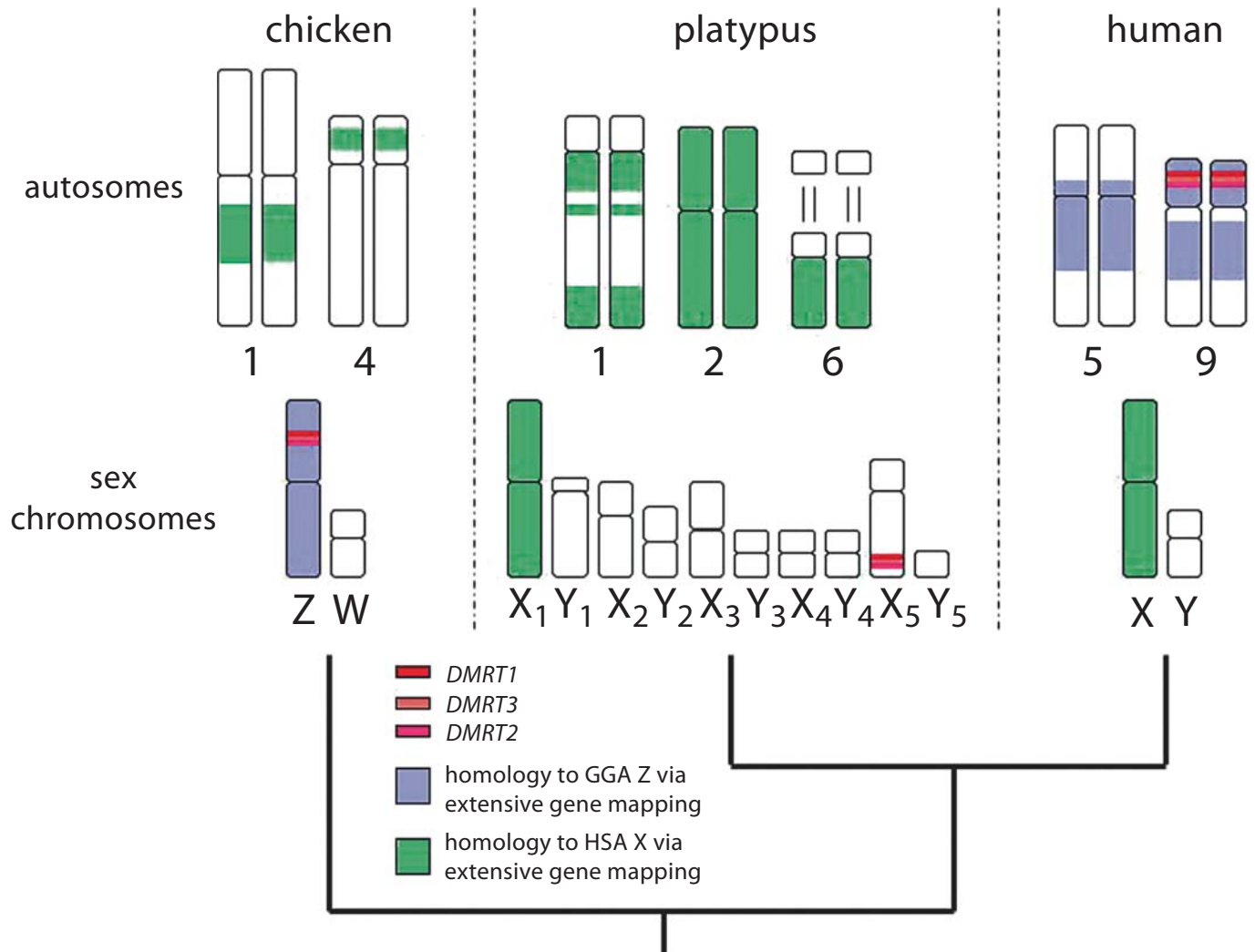


Fig. 2. Chicken (Z) and human (X) sex chromosome homology to the platypus meiotic sex chromosome chain suggests a link between the two systems. Human X is homologous to the largest platypus X chromosome (X_1) as well as to several platypus autosomes (chromosomes 1, 2 and 6). Chicken Z demonstrates homology with X_5 , on the opposite end of the chain. Adapted from Nanda et al. (2000), Grützner et al. (2003, 2004), Rens et al. (2004) and El-Mogharbel et al. (2006).

well as some platypus autosomes) (Grützner et al., 2003). However, *DMRT1*, the Z-linked, putative avian sex-determining gene, is found at the other end of the chain (on X_5) (Grützner et al., 2004; Rens et al., 2004; El-Mogharbel et al., 2006). *DMRT1* is part of a conserved gene complex with *DMRT2* and *DMRT3*, both of which also map to X_5 (Fig. 2). Recent mapping of platypus sex chromosomes (Rens et al., unpublished; Veyrunes et al., unpublished) suggests that many Z-linked genes lie on the platypus sex chromosomes. Further comparative gene mapping with other Z-linked genes would clarify this picture.

Intriguingly, *SRY*, the Y-linked mammalian testes-determining gene, has not been found in platypus (Grützner et al., 2004), so some other sex-determining gene must act as the testes-determining factor. Although *DMRT1* would be

an obvious candidate, its function would be paradoxical compared to birds, as male platypuses would receive only one copy (X_5Y_5), and female platypuses two (X_5X_5). Unless platypus sex is determined by an as yet unidentified gene, it is possible that *DMRT1* works in a novel, non-dosage-related way.

The location of *DMRT1* to a sex chromosome in the platypus, whether it plays an important role in sex determination or not, links the mammalian and avian sex chromosome systems, and suggests that the ancestral mammal might have possessed a ZZ/ZW system similar to that of birds. The mechanisms of how the 5X/5Y chain evolved from a ZZ/ZW system are reviewed in Ezaz et al. (2006).

This study therefore provides challenges to the accepted hypothesis that the mammalian XX/XY system and the avi-

an ZZ/ZW system evolved independently from two separate autosomal pairs in a reptilian ancestor with temperature-dependant sex determination (Graves, 1995; Fridolfsson et al., 1998); reviewed in Ezaz et al. (2006).

Conclusion

The ruling hypothesis for the evolution of avian and mammal sex chromosomes is poetically simple – avian and mammalian sex chromosomes evolved independently, but in parallel, from different ancestral autosomes, and the rules of progressive differentiation of the heteromorphic partner ensured the subsequent degradation of one of the sex chromosomes; the W in birds, the Y in mammals.

Only recently has modern biotechnology provided techniques, such as cross-species chromosome painting and comparative gene sequence analysis, appropriate for exam-

ining in detail these hypotheses. In addition, it was not until more exotic species could be studied, like the Californian condor, the Harpy eagle and the platypus, that we could put together a broader picture of vertebrate sex chromosome evolution, demonstrating the significance of non-model organisms in developing and analyzing important biological models and hypotheses.

These new techniques have raised further questions about the process of vertebrate sex chromosomes. Sequence comparisons between paralogous genes on the Z and W show that ZW differentiation (like XY differentiation in mammals) proceeded in fits and starts, and that differentiated Z/W chromosomes represent a converged state rather than an ancestral one. Of the greatest interest is that these new techniques, combined with work on some exotic material (condors, emus, platypus), pose some challenges to the central hypothesis that mammalian and avian sex chromosomes evolved independently.

References

- Agate RJ, Choe M, Arnold AP: Sex differences in structure and expression of the sex chromosome genes *CHD1Z* and *CHD1W* in zebra finches. *Mol Biol Evol* 21:384–396 (2004).
- Backstrom N, Cepplitis H, Berlin S, Ellegren H: Gene conversion drives the evolution of *HINTW*, an ampliconic gene on the female-specific avian W chromosome. *Mol Biol Evol* 22:1992–1999 (2005).
- de Oliveira EH, Habermann FA, Lacerda O, Sbalqueiro IJ, Wienberg J, Muller S: Chromosome reshuffling in birds of prey: the karyotype of the world's largest eagle (Harpy eagle *Harpia harpyja*) compared to that of the chicken (*Gallus gallus*). *Chromosoma* 114:338–343 (2005).
- Derjushva S, Kurganova A, Habermann F, Gaginskaya E: High chromosome conservation detected by comparative chromosome painting in chicken, pigeon and passerine birds. *Chromosome Res* 12:715–723 (2004).
- Ellegren H: Dosage compensation: do birds do it as well? *Trends Genet* 18:25–28 (2002).
- El-Mogharbel N, Wakefield M, Deakin JE, Tsend-Ayush E, Grützner F, et al: DMRT gene cluster analysis in the platypus: New insights into genomic organization and regulatory regions. *Genomics* 89:10–21 (2006).
- Ezaz T, Stiglec R, Veyrunes F, Marshall Graves JA: Relationships between vertebrate ZW and XY sex chromosome systems. *Curr Biol* 16:R736–R743 (2006).
- Fridolfsson AK, Cheng H, Copeland NG, et al: Evolution of the avian sex chromosomes from an ancestral pair of autosomes. *Proc Nat Acad Sci USA* 95:8147–152 (1998).
- Graves JA: The evolution of mammalian sex chromosomes and the origin of sex determining genes. *Phil Trans R Soc London, Series B Biol Sci* 350:305–311; discussion 311–312 (1995).
- Graves JA: Sex and death in birds: a model of dosage compensation that predicts lethality of sex chromosome aneuploids. *Cytogenet Genome Res* 101:278–282 (2003).
- Graves JA: Sex chromosome specialization and degeneration in mammals. *Cell* 124:901–914 (2006).
- Graves JAM, Shetty S: The evolution of sex chromosomes in higher vertebrates, in Clark M (ed): *Comparative Genomics*, pp 153–205 (Kluwer Academic Publishers, Boston 2000).
- Grützner F, Deakin J, Rens W, El-Mogharbel N, Marshall Graves JA: The monotreme genome: a patchwork of reptile mammal and unique features? *Comp Biochem Physiol A Mol Integr Physiol* 136:867–881 (2003).
- Grützner F, Rens W, Tsend-Ayush E, El-Mogharbel N, O'Brien PC, et al: In the platypus a meiotic chain of ten sex chromosomes shares genes with the bird Z and mammal X chromosomes. *Nature* 432:913–917 (2004).
- Guttenbach M, Nanda I, Feichtinger W, Masabanda JS, Griffin DK, Schmid M: Comparative chromosome painting of chicken autosomal paints 1–9 in nine different bird species. *Cytogenet Genome Res* 103:173–184 (2003).
- Handley LJ, Cepplitis H, Ellegren H: Evolutionary strata on the chicken Z chromosome: implications for sex chromosome evolution. *Genetics* 167:367–376 (2004).
- Hillier LW, Miller W, Birney E, et al: Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* 432:695–716 (2004).
- Hori T, Asakawa S, Itoh Y, Shimizu N, Mizuno S: *Wpkci* encoding an altered form of PKCI is conserved widely on the avian W chromosome and expressed in early female embryos: implication of its role in female sex determination. *Mol Biol Cell* 11:3645–3660 (2000).
- Itoh Y, Arnold AP: Chromosomal polymorphism and comparative painting analysis in the zebra finch. *Chromosome Res* 13:47–56 (2005).
- Itoh Y, Kampf K, Arnold AP: Sex chromosomes of the zebra finch: FISH mapping of Z chromosome genes and identification of a W chromosome specific repetitive sequence. Fourth International Symposium on the Biology of Vertebrate Sex Determination, Royal Kona Resort, Kona Hawaii USA (2006).
- Karolchik D, Baertsch R, Diekhans M, Furey TS, Hinrichs A, et al: The UCSC Genome Browser Database. *Nucleic Acids Res* 3:51–54 (2003).
- Kasai F, Garcia C, Arruga MV, Ferguson-Smith MA: Chromosome homology between chicken (*Gallus gallus domesticus*) and the red-legged partridge (*Alectoris rufa*): evidence of the occurrence of a neocentromere during evolution. *Cytogenet Genome Res* 102:326–330 (2003).
- Kettlewell JR, Raymond CS, Zarkower D: Temperature-dependent expression of turtle *Dmrt1* prior to sexual differentiation. *Genesis* 26:174–178 (2000).
- Kuroiwa A, Yokomine T, Sasaki H, Tsuzuki M, Tanaka K, et al: Biallelic expression of Z-linked genes in male chickens. *Cytogenet Genome Res* 99:310–314 (2002).
- Masabanda JS, Burt DW, O'Brien PC, et al: Molecular cytogenetic definition of the chicken genome: the first complete avian karyotype. *Genetics* 166:1367–1373 (2004).
- McQueen HA, McBride D, Miele G, Bird AP, Clinton M: Dosage compensation in birds. *Curr Biol* 11:253–257 (2001).
- Nanda I, Schmid M: Conservation of avian Z chromosomes as revealed by comparative mapping of the Z-linked aldolase B gene. *Cytogenet Genome Res* 96:176–178 (2002).
- Nanda I, Sick C, Munster U, Kaspers B, Scharl M, et al: Sex chromosome linkage of chicken and duck type I interferon genes: further evidence of evolutionary conservation of the Z chromosome in birds. *Chromosoma* 107:204–210 (1998).
- Nanda I, Zend-Ajusich E, Shan Z, 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).
- O'Neill M, Binder M, Smith C, Andrews J, Reed K, et al: ASW, a gene with conserved avian W-linkage and female specific expression in chick embryonic gonad. *Dev Genes Evol* 210:243–249 (2000).
- Ogawa A, Solovei I, Hutchison N, Saitoh Y, Ikeda JE, et al: Molecular characterization and cytological mapping of a non-repetitive DNA sequence region from the W chromosome of chicken and its use as a universal probe for sexing carinatae birds. *Chromosome Res* 5:93–101 (1997).

- Ogawa A, Murata K, Mizuno S: The location of Z- and W-linked marker genes and sequence on the homomorphic sex chromosomes of the ostrich and the emu. *Proc Natl Acad Sci USA* 95: 4415–4418 (1998).
- Oguma K: Studies on sauropsid chromosomes. *V. Annot Zool Japan* 17:612–622 (1938).
- Ohno S: Sex chromosomes and sex-linked genes. (Springer, New York 1967).
- Ohno S, Stenius C, Christian LC, Becak W, Becak ML: Chromosomal uniformity in the avian subclass Carinatae. *Chromosoma* 15:280–288 (1964).
- Oreal E, Mazaud S, Picard JY, Magre S, Carre-Eusebe D: Different patterns of anti-Mullerian hormone expression as related to *DMRT1*, *SF-1*, *WT1*, *GATA-4*, *Wnt-4* and *Lhx9* expression in the chick differentiating gonads. *Dev Dyn* 225: 221–232 (2002).
- Raudsepp T, Houck ML, O'Brien PC, Ferguson-Smith MA, Ryder OA, Chowdhary BP: Cytogenetic analysis of California condor (*Gymnogyps californianus*) chromosomes: comparison with chicken (*Gallus gallus*) macrochromosomes. *Cytogenet Genome Res* 98:54–60 (2002).
- Raymond CS, Kettlewell JR, Hirsch B, Bardwell VJ, Zarkower D: Expression of *Dmrt1* in the genital ridge of mouse and chicken embryos suggests a role in vertebrate sexual development. *Dev Biol* 215:208–220 (1999).
- Raymond CS, Murphy MW, O'Sullivan MG, Bardwell VJ, Zarkower D: *Dmrt1*, a gene related to worm and fly sexual regulators, is required for mammalian testis differentiation. *Genes Dev* 14:2587–2595 (2000).
- Reed KJ, Sinclair AH: *FET-1*: a novel W-linked female specific gene up-regulated in the embryonic chicken ovary. *Mech Dev* 119; Suppl 1:S87–90 (2002).
- Rens W, Grützner F, O'Brien PC, Fairclough H, Graves JA, Ferguson-Smith MA: Resolution and evolution of the duck-billed platypus karyotype with an X₁Y₁X₂Y₂X₃Y₃X₄Y₄X₅Y₅ male sex chromosome constitution. *Proc Natl Acad Sci USA* 101:16257–16261 (2004).
- Schmid M, Nanda I, Guttenbach M, et al: First report on chicken genes and chromosomes 2000. *Cytogenet Cell Genet* 90:169–218 (2000).
- Schmid M, Nanda I, Hoehn H, et al: Second report on chicken genes and chromosomes 2005. *Cytogenet Genome Res* 109:415–479 (2005).
- 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).
- Shetty S, Kirby P, Zarkower D, Graves JA: DMRT1 in a ratite bird: evidence for a role in sex determination and discovery of a putative regulatory element. *Cytogenet Genome Res* 99:245–251 (2002).
- Shibata K, Takase M, Nakamura M: The Dmrt1 expression in sex-reversed gonads of amphibians. *Gen Comp Endocrinol* 127:232–241 (2002).
- Shibusawa M, Minai S, Nishida-Umehara C, Suzuki T, Mano T, et al: A comparative cytogenetic study of chromosome homology between chicken and Japanese quail. *Cytogenet Cell Genet* 95:103–109 (2001).
- Shibusawa M, Nishida-Umehara C, Masabanda J, Griffin DK, Isobe T, Matsuda Y: Chromosome rearrangements between chicken and guinea fowl defined by comparative chromosome painting and FISH mapping of DNA clones. *Cytogenet Genome Res* 98:225–230 (2002).
- Shibusawa M, Nishibori M, Nishida-Umehara C, Tsudzuki M, Masabanda J, et al: Karyotypic evolution in the Galliformes: an examination of the process of karyotypic evolution by comparison of the molecular cytogenetic findings with the molecular phylogeny. *Cytogenet Genome Res* 106:111–119 (2004a).
- Shibusawa M, Nishida-Umehara C, Tsudzuki M, Masabanda J, Griffin DK, Matsuda Y: A comparative karyological study of the blue-breasted quail (*Coturnix chinensis* Phasianidae) and California quail (*Callipepla californica* Odonotophoridae). *Cytogenet Genome Res* 106:82–90 (2004b).
- Smith CA, McClive PJ, Western PS, Reed KJ, Sinclair AH: Conservation of a sex-determining gene. *Nature* 402:601–602 (1999).
- Solari AJ: Sex Chromosomes and Sex Determination in Vertebrates. (CRC Press, Boca Raton 1993).
- Stiglec R, Ezaz T, Graves JAM: Re-assignment of chicken W chromosome sequences to the Z chromosome by fluorescence in situ hybridization (FISH). *Cytogenet Genome Res* 116:132–134 (2007).
- Suzuki K: On the chromosomes of the domestic fowl. *Zool Mag (Japan)* 42:358–359 (1930).
- Takagi N, Sasaki M: A phylogenetic study of bird karyotypes. *Chromosoma* 46:91–120 (1974).
- Takagi N, Itoh M, Sasaki M: Chromosome studies in four species of ratitae (aves). *Chromosoma* 36:281–291 (1972).
- Toder R, Wakefield MJ, Graves JA: The minimal mammalian Y chromosome – the marsupial Y as a model system. *Cytogenet Cell Genet* 91: 285–292 (2000).
- Yamada D, Koyama Y, Komatsubara M, et al: Comprehensive search for chicken W chromosome-linked genes expressed in early female embryos from the female-minus-male subtracted cDNA macroarray. *Chromosome Res* 12:741–754 (2004).
- Yamashina Y: Karyotype studies in birds. *I. Cytologia* 12:270–296 (1944).