Chromosome Res (2012) 20:57–69
DOI 10.1007/s10577-011-9255-y

The sex-specific region of sex chromosomes in animals and plants

Andrea R. Gschwend · Laura A. Weingartner · Richard C. Moore · Ray Ming

Published online: 22 November 2011

Abstract Our understanding of the evolution of sex chromosomes has increased greatly in recent years due to a number of molecular evolutionary investigations in divergent sex chromosome systems, and these findings are reshaping theories of sex chromosome evolution. In particular, the dynamics of the sex-determining region (SDR) have been demonstrated by recent findings in ancient and incipient sex chromosomes. Radical changes in genomic structure and gene content in the male specific region of the Y chromosome between human and chimpanzee indicated rapid evolution in the past 6 million years, defying the notion that the pace of evolution in the SDR was fast at early stages but slowed down overtime. The chicken Z and the human X chromosomes appeared to have acquired testis-expressed genes and expanded in intergenic regions. Transposable elements greatly contributed to SDR expansion and aided the trafficking of genes in the SDR and its X or Z counterpart through retrotransposition. Dosage compensation is not a destined consequence of sex chromosomes as once thought. Most X-linked microRNA genes escape silencing and are expressed in testis. Collectively, these findings are challenging many of our preconceived ideas of the evolutionary trajectory and fates of sex chromosomes.

Keywords Dosage compensation · gene trafficking · microRNA · transposable elements · sex chromosome evolution

Abbreviations

BAC	Bacterial artificial chromosome
FSW	Female-specific region of the W chromosome
HSY	Hermaphrodite-specific region of the Y chromosome
kb	Kilobase
L1	LINE1
LG	Linkage group
LINE	Long interspersed elements
LTR	Long terminal repeat
Mb	Megabase
MiRNA	MicroRNA
MSCI	Meiotic sex chromosome inactivation
MSY	Male-specific region of the Y chromosome
NBS-LRR	Nucleotide-binding site/leucine rich repeat

Responsible Editors: Tariq Ezaz and Jennifer Graves.

A. R. Gschwend · R. Ming (✉)
Department of Plant Biology,
University of Illinois at Urbana-Champaign,
Urbana, IL 61801, USA
e-mail: rming@life.illinois.edu

L. A. Weingartner
Department of Biology, Indiana University,
Bloomington, IN 47405, USA

R. C. Moore
Department of Botany, Miami University,
Oxford, OH 45056, USA

PAM	Paternal antagonism model
PAR	Pseudo-autosomal region
PMSC	Post meiotic sex chromatin
SDR	Sex-determining region
SNP	Single-nucleotide polymorphism
TE	Transposable elements
XCI	X chromosome inactivation

The enormous diversity of life forms on earth has been driven in part by the processes of sex and recombination that evolved in single-celled organisms and amplified in multicellular organisms. The primary advantage of sexual reproduction is increased genetic diversity proposed by Weismann (1889), and it was validated by empirical data more than a century later (Goddard et al. 2005; Paland and Lynch 2006). The ultimate form of sexual reproduction that maximizes genetic diversity is dioecy in plants or gonochorism in animals, which is evolved by random mutation resulting in male-sterile or female-sterile phenotypes in hermaphroditic progenitors at least in plants (Charlesworth and Charlesworth 1978; Charlesworth 1991). Some dioecious species are controlled by sex determination genes in the autosomes and others by sex chromosomes. Those species with autosomal sex determination genes could acquire reverse mutations and change back to hermaphroditic forms. Others with sex chromosomes at advanced stages would remain dioecious as long as the sex chromosomes exist (Ming et al. 2011).

Recent findings in emerging sex chromosomes in flowering plants and fish validated their origin from autosomes (Liu et al. 2004; Peichel et al. 2004; Yin et al. 2008; Spigler et al. 2008). Sex chromosomes might evolve from two closely linked sex determination genes or two linked genes, one for sex determination and the other with a sex-specific function (Charlesworth and Charlesworth 1978). Suppression of recombination at the two linked genes is the pivotal event of sex chromosome evolution. Once the recombination is suppressed, the sex-determining region (SDR) starts accumulating retrotransposons and other repetitive sequences, degenerating gene content, and expanding the SDR region. There are three major types of sex chromosomes: XY, ZW, and UV (Bachtrog et al. 2011). Using plants as examples, the XY system evolved from a recessive mutation of a stamen-promoting gene that resulted in an intermediate gynodioecious (female and hermaphrodite) population, and a second gain of function mutation that occurred on the same chromosome, in close proximity to the functional stamen-promoting gene, which resulted in carpel suppression (Charlesworth and Charlesworth 1978). The XY system is male heterogametic and Y is the dominant sex-determining chromosome. In the ZW system, females are heterogametic and the W is the dominant sex-determining chromosome. In plants, the female specifying W contains a dominant male sterility locus permanently linked to a dominant female-promoting locus. Whether female heterogamety in plants evolves through an androdioecious or a gynodioecious intermediate depends on the nature of the first mutation. The UV system evolved in algae and bryophytes with a predominant haploid phase in their life cycle. Females, which make large gametes, are determined by a U chromosome while males, which make small gametes, are determined by a V chromosome. The diploid stage with UV chromosomes is therefore always heterogametic (Bachtrog et al. 2011).

Sex chromosomes in plants and animals and mating type loci in fungi share strikingly similar genomic features and evolutionary processes, and this convergent evolution across kingdoms reflects similar selection forces that drive their formation (Fraser et al. 2004). Sex chromosomes at advanced stages are easily distinguishable from autosomes because they are heteromorphic from each other and from the autosomes. The size variation between the pair is caused by different rates of expansion or contraction of the sex-limited, hemizygous chromosome (Y or W) compared to their counterpart (X or Z). The dynamics of sex chromosome evolution are limited to the non-recombining SDR and its X or Z counterpart, whereas the pseudo-autosomal regions recombine normally like autosomes, despite elevated recombination rates near the borders of SDR (Yu et al. 2009). In this review, we focus on recent advances in the genomics of SDR and its X or Z counterpart, because of their defining role in sex determination and impact on human health and crop improvement. The term SDR is used as an equivalent to the male-specific region of the Y chromosome (MSY), the hermaphrodite-specific region of the Y chromosome (HSY) in trioecious species with two Y chromosomes (male Y and

hermaphrodite Y), and the female-specific region of the W chromosome (FSW).

Molecular dynamics of the sex-determining region

A prominent feature of the SDR is its variation in size compared to its X or Z counterpart at various stages of sex chromosome evolution (Ming et al. 2011). At the earliest stage when there is no suppression of recombination around the two sex determination/sex-specific loci, the prototype sex chromosomes are like homologous autosomes with no difference in size. Once the SDR is suppressed for recombination, it accumulates retrotransposable elements and duplicated sequences, initiating an expansion phase (Fig. 1). The poplar W chromosome is 706 kb (5.9%) longer than the 11.3-Mb Z chromosome (linkage group (LG) 19) (Tuskan et al. 2006; Yin et al. 2008). Based on the relative length of pachytene chromosomes, the papaya X chromosome is about 46 Mb, while the papaya Y chromosome is 4.6 Mb (10%) longer than the X chromosome (Zhang et al. 2010a; Na et al. 2012). The most dramatic example of the expansion phase is the 570-Mb Y chromosome compared to the 420-Mb X chromosome in *Silene latifolia* (Liu et al. 2004). The degree of SDR expansion is generally positively correlated to the time of suppression of the SDR and sex chromosome divergence within a particular lineage of organisms in the early stages of sex chromosome evolution, though ratite birds, with evolutionarily old ZW chromosomes, have a relatively small SDR (Tsuda et al. 2007; Charlesworth and Mank 2010). The papaya sex chromosomes evolved about 2–3 Ma ago (Mya) (Yu et al. 2008), whereas the sex chromosomes of *S. latifolia* evolved about 10 Mya (Bergero et al. 2007). The human sex chromosomes are ancient and evolved about 166 Mya; they are at an advanced evolutionary stage and the Y chromosome has likely already gone through the expansion phase and is currently at the contraction phase (Veyrunes et al. 2008). Thus, the Y chromosome is about 66 Mb compared to the 155 Mb of the X chromosome (Skaletsky et al. 2003; Ross et al. 2005). The human and chimpanzee Y chromosomes shared a common ancestral Y chromosome for 160 of the 166 Ma, but, after 6 Ma of divergence, the euchromatic region of the MSY in chimpanzee is 3 Mb (13.2%) larger than that of the human MSY (Hughes et al. 2010).

In medaka fish, the sex determination gene *dmrt1bY* was duplicated from the ancestral *dmrt1a* gene on LG9 and translocated to the Y chromosome. This duplication and translocation event was estimated to have occurred about 10 Mya, but the Y chromosome contained a 258-kb MSY, much smaller than that of the more recently evolved papaya Y chromosome (Kondo et al. 2004, 2006). The small size of the medaka MSY is likely restricted by the unique structure of the flanking borders, where duplicated genes *OlaflnkL* and *OlaflnkR* are located.

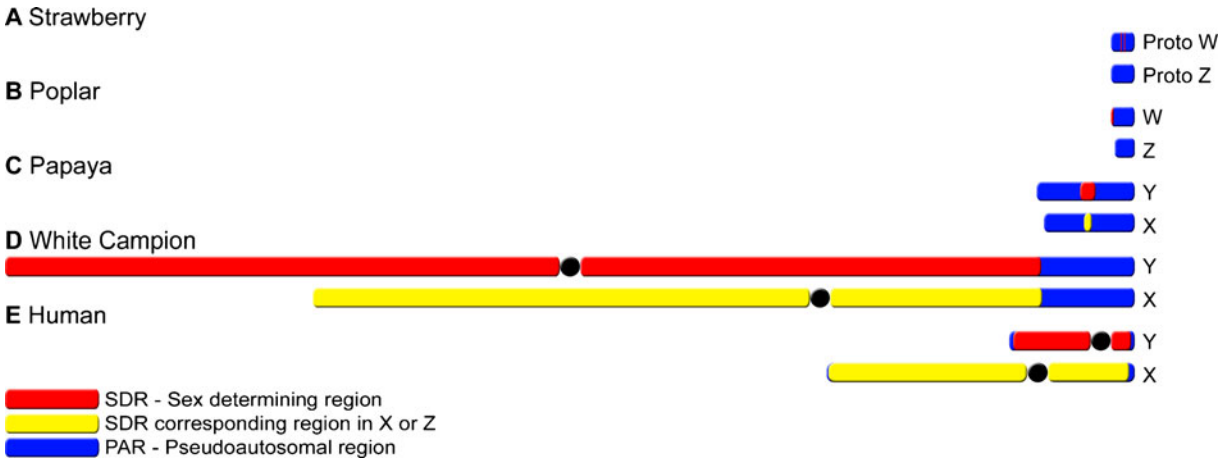

Fig. 1 Comparative organization of SDR in selected species. The variation of size is a dynamic process at different stages of sex chromosome evolution. The two vertical lines in the proto W chromosome represent the two sex determination genes. The estimated sizes of sex chromosomes are: *A* Strawberry, Z=W=~12 Mb; *B* Poplar, Z=11.3 Mb; W=12 Mb; *C* Papaya, X=~46 Mb, Y=~50 Mb; *D Silene latifolia*, X=~420 Mb, Y=~570 Mb; *E* Human, X=155 Mb, Y=66 Mb

Either of these two genes can recombine with the single copy X counterpart *OlaflnkX*. The medaka MSY consists of a 72.1-kb Y core region that contains the sex determination gene *dmrt1bY*, and two bordering regions with 12.2 and 63.4 kb, respectively. The 72.1-kb Y core region is aligned with 42.9 kb of LG9 that contains the ancestral *dmrt1a* gene, showing 68% sequence expansion.

Chromosomal rearrangements are also dynamic in the SDR. Inversions are often the molecular basis of recombination suppression. Five evolutionary strata in the human X chromosome were created by five inversion events on the Y chromosome, although there is no direct evidence except the pericentric inversion shown in elephant X chromosome (Lahn and Page 1999; Ross et al. 2005; Delgado et al. 2009). Three evolutionary strata triggered by three progressive inversions are also found in the chicken Z chromosome (Nam and Ellegren 2008). The eight palindromes in the human MSY are inverted duplications, and such structure has not been found in the recently evolved poplar W and papaya Y chromosomes (Yin et al. 2008; R. Ming unpublished data). There are 19 palindromes in the chimpanzee MSY (Hughes et al. 2010). Among them, seven are shared with the human MSY, perhaps inherited from the ancestral Y chromosome, and 12 are chimpanzee-specific. Moreover, most palindromes in the chimpanzee MSY exist in multiple copies, resulting in a higher rate of arm-to-arm sequence divergence in some chimpanzee palindromes compared to that of single-copy palindromes in the human MSY. Small-scale local rearrangements were hard to find in the human X and Y since the Y chromosome had gone through the degeneration processes with hardly any traces of local rearrangement remaining. Such local rearrangements were numerous between the X and Y chromosomes in papaya, including inversions, insertions, deletions, duplications, and translocation (Yu et al. 2008; R. Ming unpublished). Inversions and deletions were also found in the Y chromosome of three-spined stickleback fish (Ross and Peichel 2008). Numerous chromosomal rearrangements were found between human and chimpanzee Y chromosomes, whereas the human and chimpanzee chromosome 21 were collinear (Hughes et al. 2010).

The rapid divergence of the SDR offers an opportunity to test the existence of sex chromosomes in dioecious species using next-generation sequencing technologies. For instance, it has been controversial whether sex chromosomes have evolved in dioecious date palm. Three male and six female date palm genomes were sequenced using Illumina with sequence coverage ranging from 10.1 times to 53.4 times (Al-Dous et al. 2011). Single-nucleotide polymorphism (SNP) analysis revealed that 1,605 out of 3.5 million SNPs segregated with sex, and 923 (58%) of them were in 24 scaffolds spanning 602 kb. Heterozygous SNP genotypes were found in all male genomes and homozygous genotypes in all female genomes, indicating that date palm indeed has XY chromosomes. Genetic mapping of SNPs in the four scaffolds containing the largest number of SNPs showed that all four scaffolds are linked with no recombination. This approach could be used to quickly analyze the SDRs of dioecious species with limited genomic resources.

Loss and gain of genes in the sex-determining region

To study the loss and gain of genes in the SDR, complete sequencing of the SDR and its X or Z counterpart would provide the ultimate resolution. However, nearly all *de novo* genome sequencing projects were carried out using the homogametic sex to avoid the difficulty of assembling the heterozygous SDR region, although heterogametic sex genotypes were used for re-sequencing projects. The only exception is the poplar genome sequencing project that used a female plant, but later discovered that poplar has a ZW sex chromosome system (Tuskan et al. 2006; Yin et al. 2008). Currently, sequencing of the SDR and its X or Z counterparts is complete for human, medaka fish, poplar, and papaya (Skaletsky et al. 2003; Ross et al. 2005; Kondo et al. 2006; Tuskan et al. 2006; R. Ming unpublished data).

The most extensively studied sex chromosomes are those in human. The human Y chromosome lost most of the 1,098 genes present in the X chromosome (Ross et al. 2005). The MSY contains 78 protein coding genes that encode 27 different proteins (Skaltsky et al. 2003). Out of the 78 genes, only 16 (20.5%) genes were likely remnants of the original set of genes in the ancestral autosomes, and these 16 genes shared homology with 14 single-copy genes on the X chromosome, including two pairs of genes on

the Y corresponding to two single-copy genes on the X, which could have resulted from duplication events in the Y chromosome or the loss of one gene of the ancestral pairs in the X chromosome. The paired genes on the Y have diverged, and each encodes a different protein. These 16 X-degenerated genes encode 16 proteins. Two of the 78 MSY genes were transposed from the X chromosome about 3–4 Mya, encoding two proteins. The remaining 60 genes are in the eight palindrome sequences, and some have orthologs in the X chromosomes, such as RBMX (Delbridge et al. 1999). These 60 ampliconic genes are in 9 gene families and encode 9 distinctive proteins that are expressed in the testis. Moreover, the human MSY includes 78 additional transcription units without strong evidence of being protein coding, including 13 single-copy units and 65 units in 15 MSY-specific families. Taken together, human MSY gained 125 (80.1%) of the detectable 156 transcription units.

The complete sequencing of the chimpanzee Y chromosome provided a second well-characterized mammalian Y chromosome to examine the loss and gain of genes within a 6 million year time frame (Hughes et al. 2010). The chimpanzee MSY contains 37 genes coding for 18 different proteins, including 12 X-degenerated and 25 ampliconic genes. The chimpanzee MSY lost four X-degenerated genes that are in human within 6 Ma. This is further confirmed by the presence of all 16 genes in the gorilla Y chromosome that shared a common ancestor with the human–chimpanzee lineage about 7 Mya (Goto et al. 2009). It is not clear whether gorilla retains additional X-degenerated genes that are lost in the human MSY since the gorilla Y chromosome has not been fully sequenced. Chimpanzee also lost 3 of the 9 gene families by frameshift mutations, albeit it has 11 more palindromes than those in human, with 25 genes in 6 gene families remaining. No new ampliconic genes are found in chimpanzee, suggesting that the nine ampliconic gene families were likely acquired from autosomes before the divergence of human and chimpanzee. One gene family, *TSPY*, has 35 members in human, but only 6 in chimpanzee, perhaps due to expansion in human. Of course, chimpanzee is lacking the two X-transposed genes acquired by the human MSY 3–4 Mya after their split. Although the human MSY appears to have limited gene loss, if any, in 6 Ma in comparison with the chimpanzee MSY, four new genes were found in the cat MSY; two are X-degenerated and the other two are originated from autosomes (Murphy et al. 2006). Since cat and human diverged about 95 Mya, the two X-degenerated genes are likely lost in the human lineage, but the two genes acquired from autosomes could be lost in the human or gained after their divergence (Springer et al. 2003).

The gene loss in the human MSY is so extensive that it is difficult to assess how many of the 1,098 genes in the X chromosomes are from the ancestral autosomes. Young sex chromosomes in flowering plants and fish fill in the gap to document the gene gains and losses of the SDR in the early stage of sex chromosome evolution. The medaka Y chromosome, for example, has an MSY restricted in a small 258-kb region because of the pair of duplicated genes in the borders, formed by an insertion of a duplicated fragment of LG9 into the current Y chromosome, thus having no homologous sequence in the X chromosome. The ancestral 42.9 kb sequence on LG9 contains four functional genes, including the sex determination gene paralog *dmrt1a* and one pseudogene. The MSY contains only one functional gene, the sex determination gene *dmrt1bY*, and four pseudogenes, showing the loss of three genes that are still functioning in LG9 (Kondo et al. 2006). The poplar W chromosome is also unique. It has a 706-kb SDR region with no Z counterpart and contains abundant nucleotide-binding site/leucine-rich repeat (NBS-LRR) genes, so it is not possible to assess gene gains and losses (Yin et al. 2008). Papaya sex chromosomes are more conventional, with an 8.6-Mb MSY and a 4.5-Mb X counterpart. The HSY lost 36 (33%) genes and gained 9 genes from autosomes (Ming unpublished).

The liverwort V (previously referred to as Y) chromosome was sequenced, and 64 genes were annotated in the 10-Mb V chromosome (Yamato et al. 2007). The low gene density (one gene per 156 kb) suggests gene loss in the V chromosome, but without the sequence of the U chromosome, it is hard to assess the extent of degeneration in the V chromosome. Nevertheless, 14 of the 64 genes are V-specific, indicating that gene gains have occurred in the liverwort V chromosome.

Gene gain and loss in the X/Z chromosome

The X chromosome has been thought to remain relatively stagnant and to conserve the structure of the autosome from which the sex chromosomes

originally arose (Ohno 1967; Bull 1983). Recently, additional findings on the X chromosome have brought to light a more complex scenario. Over time, the X chromosome has been altered and shaped by selection pressures, creating a distinctly different chromosome than its ancestral counterpart.

One such way, the X chromosome has evolved is by gene trafficking. The therian and *Drosophila* X chromosomes both selectively lost genes, many of which function during meiosis, as well as gained sex-biased genes (Potrzebowski et al. 2008, 2010; Vibranovski et al. 2009). The young X chromosome went through increased bouts of adaptive evolution and demasculinization of its gene content (Bachtrog et al. 2009). In mammals, retrotransposed copies of important housekeeping genes were fixed into the autosome, possibly as a way to compensate for the X-linked parental gene being silenced during and after meiosis through meiotic sex chromosome inactivation (MSCI) (Potrzebowski et al. 2008). MSCI is the heterochromatinization of the X and Y chromosomes during meiosis, which silences transcription. Silencing crucial housekeeping genes on the X could be detrimental, but autosomal retrotransposed copies of the vital silenced X genes compensate for that loss. This type of adaptive evolution is also seen in *Drosophila*, where meiotic testis-expressed genes were retrotransposed from the X chromosome to the autosome, suggesting MSCI may influence gene content on the X chromosome (Vibranovski et al. 2009).

The newly formed X chromosome was also affected by adaptive evolution in that it gained genes. A study by Zhang et al. (2010b) found two peaks in gene gain in the mammalian X chromosome. The first gene burst occurred before the split of eutherian mammals, around the time the X chromosome was beginning the transformation from autosome to sex chromosome. The newly evolving X chromosome was subjected to increased positive selection and quickly accumulated sex-biased retrogenes, many of which were female-biased, having higher expression in the ovaries (Potrzebowski et al. 2010; Zhang et al. 2010b). This enhanced positive selection was also observed in the neo-X chromosome of *Drosophila* (Bachtrog et al. 2009). It appears that sex-related selection played a large role in shaping the gene content of the nascent X chromosome.

The second burst of gene gain on the X chromosome occurred recently, seen both after the split of human and chimpanzee and after the split of mouse and rat (Zhang et al. 2010b). Fisher hypothesized that male-advantage genes, that are detrimental in females, will accumulate on the X chromosome (Fisher 1931). These male-advantage female-detrimental genes are dominant in males and recessive in females and eventually evolve male-specific expression (Fisher 1931). The younger genes acquired by the X chromosome during the second gene burst are often male-biased genes that were likely fixed onto the X chromosome through sexual antagonism, supporting Fisher's hypothesis (Zhang et al. 2010b). These newly gained genes are present in multiple copies in the X chromosome and are surrounded by repeats. Interestingly, many of these new male-biased genes are not silenced during meiosis or post-meiosis, suggesting that they are not affected by MSCI (Zhang et al. 2010b). Both human and mouse X chromosomes were found to have young male-biased genes with testis-biased expression. The human X chromosome gained multicopy cancer/testis antigen gene families, which are predominantly expressed in the testis (Ross et al. 2005). The mouse X chromosome has X-ampliconic multicopy genes with testis-biased expression, most of which were found to be specifically expressed post-meiotically (Mueller et al. 2008). Multiple copies of these genes amplify the gene expression, allowing for these genes to function even when faced with partial X chromosome repression (Mueller et al. 2008). It is thought that the inverted repeats surrounding the male-biased multicopy genes in human and mouse protect the genes from MSCI through unusual chromatin formations (Warburton et al. 2004). This "strategy" to avoid MSCI may have allowed the accumulation of sex-biased genes on the X-chromosome to once again be beneficial.

There is also evidence of Z-chromosome divergence from ancestral autosomes. The mammalian X chromosome and avian Z chromosome evolved from two different parts of the ancestral genome, but the X and Z chromosomes have progressed similarly through convergent evolution (Bellott et al. 2010). The chicken Z chromosome also gained a substantial number of genes compared to its orthologous autosome, the majority of which are multicopy genes (Bellott et al. 2010). The chicken Z chromosome contains a "Z amplicon," a tandem array of genes expressed predominantly in the testis (Bellott et al.

2010). It is interesting that both sex chromosome systems separately evolved an increase of male-biased genes, even though females are heterogametic in chickens. Following Fisher's logic, the opposite would be expected, where female-advantage male-disadvantage genes would accumulate on the Z and evolve female-specific expression (Fisher 1931). Instead, incomplete dosage compensation of the Z chromosome may be affecting the male-biased gene accumulation (further discussed in the Dosage compensation and sex chromosome inactivation section) (Mank 2009).

Dosage compensation and sex chromosome inactivation

According to the paradigm for the evolution of sex chromosome dosage compensation, the loss of function of genes on the sex-limited chromosome (the Y or W) due to chromosomal degeneration creates an imbalance in copy number (i.e., gene dosage) in the heterogametic sex. Consequently, there is evolutionary pressure for transcriptional upregulation of the remaining functional X- or Z-linked copy in the heterogametic sex in order to maintain balanced gene expression between sex-linked and autosomal loci (i.e., dosage compensation.). Although the hyperactivation of the functional sex chromosome is limited to the heterogametic sex in some organisms, such as *Drosophila*, in others, increased transcriptional activity occurs in the homogametic sex, as found in therian mammals (Mank 2009; Mank et al. 2011; Vicoso and Bachtrog 2009). In the latter case, dosage compensation leads to selection for the evolution of an independent mechanism that globally represses gene expression of one copy of the sex chromosome in females, known as X chromosome inactivation (XCI). In this way, XCI balances transcriptional levels of sex-linked genes among males and females. This paradigm, however, has been overturned by comparative evolutionary genomic research in recent years and it is now apparent that the evolution of global dosage compensation of sex chromosomes is the exception, not the rule (Mank et al. 2011). Consequently, this questions the origin of XCI as a compensatory mechanism for gene dosage effects; instead, it is likely that XCI evolved independently of gene dosage effects.

Studies in invertebrate model systems, such as *Drosophila* and *Caenorhabditis elegans*, as well as in mammalian models, such as humans and mice, initially hinted that dosage compensation may be a common feature of sex chromosome evolution (Mank 2009). Transcriptional upregulation of male X-linked genes in these systems was considered necessary to avoid the deleterious effects of unbalanced transcription of sex-linked genes integral to biochemical and regulatory gene networks (Birchler and Veitia 2010). However, the universality of dosage compensation is challenged by the lack of global dosage compensation in organisms with heterogametic females (ZW), including birds, silkworms, and the parasite *Schistosoma mansoni* (Itoh et al. 2007; Vicoso and Bachtrog 2011; Wolf and Bryk 2011; Zha et al. 2009). Localized upregulation of specific Z-linked genes occurs in ZW females and the ratio of global transcript levels of Z-linked genes to autosomal transcripts in ZW females is less than 1, the expected Z/A ratio if global dosage compensation were occurring (Itoh et al. 2007).

It has been hypothesized that the difference in dosage compensation mechanisms between XY and ZW systems is due to differences in sexual antagonism between male heterogametic and female heterogametic systems (Naurin et al. 2010; Mank 2009; Mank et al. 2011). Because the sex chromosomes spend disproportional amounts of time in the separate sexes, they are likely candidates for harboring sexually antagonistic genes, those that benefit one sex over the other (Naurin et al. 2010; Mank 2009). For example, the Z chromosome in female heterogametic systems spends two thirds of the time in males; as a consequence, sexual selection favors an overrepresentation of genes with male-biased gene expression on the Z chromosome, a phenomenon that has been observed in chicken (Kaiser and Ellegren 2006; Storchova and Divina 2006). According to this hypothesis, masculinization of the Z would select against global dosage compensation of the Z in females, as it would lead to an equalization of transcript levels of male beneficial genes between females and males. Alternatively, it may be that the preponderance of Z-linked genes that are overexpressed in males is not due to sexual selection, but the consequence, rather than the cause, of incomplete dosage compensation of the Z chromosome (Mank 2009). The abundance of genes with male-biased gene expression in a variety of somatic

tissues, including some housekeeping genes, on the Z supports this alternative hypothesis (Ellegren et al. 2007; Itoh et al. 2007). If sexual antagonism due to Z-linked male-biased genes limits dosage compensation in female heterogametic systems, one might expect limited dosage compensation of X-linked female-biased genes in male heterogametic systems (Mank 2009). Indeed, next-generation genomic analysis of dosage compensation has questioned the existence of global dosage compensation in some XY systems (Xiong et al. 2010). A re-analysis of sex-linked transcription in humans and mice using RNA-Seq found that expression of X-linked loci in both males and females was roughly one half that of autosomal loci (Xiong et al. 2010), challenging earlier reports of equalized X transcription using microarray analysis (Gupta et al. 2006; Nguyen and Disteche 2006). Furthermore, global dosage compensation was found to be developmentally regulated in *C. elegans* hermaphrodites, with X/A ratios decreasing to approximately 0.5 as the worms developed into adults using RNA-seq analysis (Xiong et al. 2010). It may be premature to overturn the paradigm of dosage compensation in these systems based on a single study using next-generation sequencing technology; however, it is apparent that microarray analyses are biased by the method used to filter low abundance transcripts that can lead to false interpretations of dosage compensation in these systems (Castagné et al. 2011). Of the organisms studied to date, global dosage compensation is confined primarily to dipterans, including *Drosophila* and possible *Anopheles* mosquito (Mank et al. 2011). These results also support the otherwise perplexing observation that orthologs of yeast haploinsufficient genes are significantly under-represented on mammalian and *C. elegans* X chromosomes, while they are not differentially represented on the X chromosome of *Drosophila* (Oliver et al. 2011). Because haploinsufficient genes are deleterious at decreased expression levels, selection would favor their paucity on the X in systems lacking dosage compensation, while such evolutionary pressure would not exist for systems with dosage compensation.

The discovery that dosage compensation is absent in male and female mammals challenges the long-standing idea that XCI in females evolved as a compensatory response to a hyperactive X chromosome in heterogametic males. Instead, this observation bolsters support for alternative hypotheses that suggest XCI evolved independently of dosage compensation. For example, the paternal antagonism model (PAM) posits that X-inactivation evolved as a mechanism to reduce transcript levels of paternally derived fetal growth genes that benefit the father over the mother (Engelstadter and Haig 2008; Haig 2006). According to the PAM, XCI is a form of genomic imprinting that regulates expression of genes that benefit one parent over the other (Haig 2006). Consistent with this hypothesis, XCI is limited to therian mammals with internal gestation and absent in egg-laying mammals, or monotremes (Deakin et al. 2008). Furthermore, it is the paternal X that is inactivated in marsupials (Deakin et al. 2009; Namekawa et al. 2007). This is in contrast to most eutherians in which XCI randomly affects the maternal or paternal chromosome in the developing embryo. While random XCI in eutherians seems counter to the PAM, there is a notable exception in mice. Mice have paternal imprinted XCI up until the eight-cell embryo stage, after which the silenced paternal chromosome is restricted to cells of the future placenta; in the inner cell mass, the paternal X is reactivated allowing for random inactivation later in embryonic development (Okamoto et al. 2011).

Because X inactivation of the paternal X is limited primarily to marsupials, paternal imprinting of the X is considered the ancestral condition, which is maintained to a limited degree in mice, but is lost in most other eutherians (Deakin et al. 2009; Namekawa et al. 2007). However, genomic analyses of paternal XCI in the marsupial *Monodelphis domestica* (the South American opossum) and mice provide conflicting viewpoints on the evolutionary origins of paternal imprinting in mice. A study based on Cot-1 RNA fluorescence in situ analysis supports the claim that the imprinted paternal X is the basal condition in therian mammals; the paternal X chromosome in the opossum is inactivated during meiosis and this inactive state is maintained following spermatogenesis (Namekawa et al. 2007). However, research based on transcript profiling of X-linked genes suggests that paternal imprinting in mice may be a derived characteristic (Mahadevaiah et al. 2009). While the paternal X is inactivated during spermatogenesis in both opossums and mice, this meiotic inactivation is maintained only in the mouse (Mahadevaiah et al. 2009). In the opossum, transcriptionally repressed X-linked genes are reactivated in round spermatids

to be subsequently silenced in the developing embryo, whereas in the mouse, imprinting of the paternal X is at least partially maintained and inherited by offspring (Mahadevaiah et al. 2009). It may be that XCI has evolved along separate evolutionary trajectories in marsupials and eutherians (Deakin et al. 2009). Indeed, though they share limited aspects of XCI (including, at least in some cell types, transcription-repressing histone modifications such as H3K27 trimethylation) the molecule that triggers XCI in eutherians, the non-coding RNA *XIST*, is noticeably lacking in marsupials (Chaumeil et al. 2011; Davidow et al. 2007; Hore et al. 2007; Mahadevaiah et al. 2009). Full understanding of the evolutionary basis of XCI in therian mammals may rely on the identification of the molecular trigger of XCI in marsupials, though much has been learned already from comparative genomic analyses of XCI in diverse mammalian species (Chaumeil et al. 2011; Deakin et al. 2009; Escamilla-Del-Arenal et al. 2011).

Ultimately, understanding the evolution of dosage compensation will require comparative evolutionary analyses of sex chromosome systems from all major eukaryotic lineages and at different stages of sex chromosome evolution. Little is known, for example, of the extent of dosage compensation in the relatively young sex chromosomes of plants. While early cytogenetic work in *S. latifolia* found evidence of XCI in females (Siroky et al. 1998), large-scale analysis of sex-linked transcripts in males and females is lacking. Perhaps the effects of gene dosage imbalance are of less consequence to systems such as plants and fish with frequent polyploidy and/or aneupolody (Mank et al. 2011; Vicoso and Bachtrog 2009). The application of next-generation sequencing technologies, such as RNA-Seq transcript analysis, hold promise for investigating the effects of gene dosage imbalance in such systems, in particular for the systems which lack extensive genomic resources (Wolf and Bryk 2011; Xiong et al. 2010).

Selective advantage of transposable elements

The presence of increased levels of transposable elements (TEs) is characteristically associated with degeneration in sex chromosomes. Researchers commonly use the presence of TEs on one sex chromosome and not the other (e.g., accumulation on the Y and not the X) as indirect evidence of reduced efficiency of selection. For instance, higher levels of TE insertions in the young Y chromosome of *S. latifolia* was recently reported as evidence of degeneration, as TE accumulation on the Y is thought to be responsible for intron expansion and overall chromosome enlargement as compared to the X (Marais et al. 2008). However, the understanding of TE involvement in sex chromosome evolution is being reconsidered, because of the epigenetic effects associated with these genetic elements (Slotkin and Martienssen 2007).

The epigenetic function of TEs provides a possible selective advantage during X inactivation. The role of TEs in X-chromosome inactivation was proposed over a decade ago (Lyon 1998, 2006), and recent studies have found indirect support for this hypothesis. In an analysis of L1 (or LINE1) TE interruptions on the human X chromosome, L1 elements were found to be both overrepresented and under-interrupted on the X chromosome as compared to autosomes (Abrusan et al. 2008). This trend is also stronger for L1 elements in older strata (where more silencing occurs) and for L1 elements located near genes that are inactivated, whereas L1 elements located near genes that escape inactivation are interrupted more frequently (Abrusan et al. 2008). This could be selection against knockout insertions in the L1 elements by other TEs, which indirectly supports the hypothesis that the integrity of these particular elements is perhaps important to X-chromosome inactivation (Abrusan et al. 2008). There is also evidence that the regulation of X-linked TEs may be correlated to structural polymorphisms found in the Y (Lemos et al. 2008), so it is possible that this regulation may have additional epigenetic effects in males where L1 elements are known to mobilize in the male germ line (Branciforte and Martin 1994). Comparisons of the chicken Z chromosome show that, like the mammalian X, the Z is also enriched in LINE elements (70% higher than the autosomes) (Bellott et al. 2010). This accumulation pattern suggests that LINE elements may provide some exclusive advantage to sex chromosomes. Additionally, there is evidence that an ancient retrotransposon regulates both initiation and spread of X-chromosome inactivation on the mammalian X (Cohen et al. 2007). TE accumulation appears to be a contributing factor to the evolution of sex chromosomes, and this repurposing of TEs on the X

in particular suggests that these elements serve a selective role.

In addition to a possible selective advantage for L1-mediated X inactivation within the sex chromosomes, it has also become clear that the trafficking of particular genes through retrotransposition is common in the sex chromosomes. L1 elements provide the machinery for retroduplication (Ding et al. 2006), and this mechanism is thought to allow for the movement of genes on and off the sex chromosomes. Although there is conflicting evidence for the specific type of expression restriction that occurs in *Drosophila*, support for MSCI (Hense et al. 2007) or an as-of-yet identified type of gene-expression restriction (Meiklejohn et al. 2011) in *Drosophila* underlies the idea that restricted expression is associated with the excess number of testes-specific genes that have moved from the X chromosome to an autosome. A recent analysis of therian genomes also found an insertion and retention bias of retrocopied genes on the X over other chromosomes, suggesting that some sort of selective advantage has allowed for this accumulation of retrogenes on the X (Potrzebowski et al. 2010). Additionally, this analysis showed evidence that the accumulation of retrogenes started only after sex chromosome differentiation, which implies that sex-related selection drove this enrichment of retroposed genes (Potrzebowski et al. 2010). The finding that many retrogenes escape post-meiotic silencing also suggests that there may be an advantage for these retrogenes to be expressed during this stage of spermatogenesis (Potrzebowski et al. 2010). These analyses suggest that TEs may have multiple roles in shaping sex chromosome evolution, and it is possible that they create a selective advantage through epigenetic services on the X.

miRNAs revise the role of the X chromosome in spermatogenesis

Micro-RNAs (miRNAs) are short regulatory RNAs whose crucial role in manipulating the products of protein-coding genes has been evaluated only recently (Bartel 2004). While protein-coding genes on the sex chromosomes are largely silenced during MSCI, it has recently been found that 19% of surveyed miRNA-coding genes transcribed in mouse pachytene spermatocytes are X-linked (Ro et al. 2007). This and the follow-up study found many of these X-linked miRNAs are actually testis-specific or testis-preferential (Ro et al. 2007; Song et al. 2009). The X-linked miRNAs were grouped into different types based on their expression patterns during spermatogenesis, which included miRNAs that are subject to MSCI, miRNAs that escape MSCI, but are then down-regulated in post-meiotic sex chromatin (PMSC), and miRNAs that escape both MSCI and PMSC silencing. The latter two groups make up a majority of the X-linked miRNAs analyzed (86%), suggesting that most X-linked miRNAs are actually able to escape silencing whereas most X-linked protein-coding mRNAs are not expressed (Song et al. 2009). It has been proposed that these miRNAs are involved in some sort of crucial, post-translational manipulation of proteins within the spermatocyte, but additional research will be needed to assess their exact role. These findings revolutionized the way we understand MSCI in that an entire collection of X-linked genes escape silencing during spermatogenesis. Since the transcripts were shown to be produced during and not prior to MSCI, this also indicates that some region(s) of the X chromosome must remain euchromatic during MSCI to allow for the miRNA transcription (Song et al. 2009). The lack of known Y-linked miRNAs implies that there may be an advantage for such genes to reside on the X because obligate XY heterozygosity ensures that X-linked miRNAs complement this lack of miRNA loci on the Y (Ro et al. 2007).

These findings, along with other studies that analyze protein-coding genes on the X (see Gene gain and loss in the X/Z chromosome section), have also influenced our understanding of X-chromosome demasculinization and contributions to spermatogenesis. A large number of testis-specific genes have been transposed off of the X chromosome to autosomes, probably since most of the X chromosome is silenced during MSCI (Vibranovski et al. 2009). However, these new findings that testis-specific or testis-preferential miRNAs are X-linked and that X-linked miRNAs are fully transcribed and processed in pachytene spermatocytes both suggest that some X-chromosome genes play crucial roles in spermatogenesis (Ro et al. 2007; Song et al. 2009). In fact, all of the known miRNAs transcribed from the X are expressed in the testis (Ro et al. 2007), and a survey of young X-linked miRNA transcripts found that nearly 70% are expressed at higher levels in the testes

than in several other tissues examined (Zhang et al. 2010b). This recent gain of male-biased miRNA genes on the X aligns with the pattern for protein-coding genes in which there is a bias for older X-linked genes to be expressed in the ovaries and for younger X-linked genes to be more male-biased (Zhang et al. 2010b). Considering the extensive conservation of miRNAs in evolutionary history, it will be interesting to see if a role for miRNAs becomes evident in other sex chromosome systems.

Prospects

The SDRs are dynamic systems with lineage-specific gene gains and losses that have the potential to alter the course of sex chromosome evolution in particular lineages. The complete sequencing of chimpanzee MSY, chicken Z chromosome, and the papaya HSY and its X counterpart added clarity to the genomic features and consequence of sex chromosome evolution. But so far, complete sequence data of the SDR and its counterpart are only available in human and papaya. Incomplete datasets often lead to inaccurate and sometimes wrong conclusions. It would be ideal to have complete sequences of the sex chromosomes, as well as whole genomes, across the tree of life, to make rapid progress on sex chromosome research subsequently benefiting the research and development in medicine and agriculture. Next-generation sequencing technologies make quick assessment of sex chromosomes possible as demonstrated by the identification of the XY chromosomes in date palm. But short reads and the whole genome shotgun approach yield draft genomes with numerous gaps. They are particularly ineffective for assembly the heterochromatic SDRs and their counterpart. The third-generation sequencing technologies have the potential to generate ultralong reads, close to the size of bacterial artificial chromosome (BAC), but it may take some years to reach the ultralong reads with acceptable low error rate. Before then, the costly and time-consuming BAC by BAC sequencing approach remains effective to sequence the SDRs. To reduce the cost, economically important nascent sex chromosomes in plant and fish species are good candidates for sequencing the SDRs. Such genomic resources would accelerate the identification of sex determination genes and allow for the adequate studying of gene trafficking, sex-biased gene expression, dosage compensation, epigenetic regulation, and the interaction between sex-liked genes and environment.

Acknowledgments We thank Jennifer Han for her assistance. This work was supported by a grant from the National Science Foundation (NSF) to RM, QY, PHM, and RCM (Award No. DBI-0922545) and by the National Evolutionary Synthesis Center (NESCent), NSF # EF0423641.

References

Abrusan G, Giordano J, Warburton PE (2008) Analysis of transposon interruptions suggests selection for L1 elements on the X chromosome. PLoS Genet 4:e1000172

Al-Dous EK, George B, Al-Mahmoud ME et al (2011) De novo genome sequencing and comparative genomics of date palm (*Phoenix dactylifera*). Nat Biotechnol 29:521–527

Bachtrog D, Jensen JD, Zhang Z et al (2009) Accelerated adaptive evolution on a newly formed X chromosome. PLoS Biol 7:712–719

Bachtrog D, Kirkpatrick M, Mank JE et al (2011) Are all sex chromosomes created equal? Trends Genet 27:350–357

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297

Bellott DW, Skaletsky H, Pyntikova T et al (2010) Convergent evolution of chicken Z and human X chromosomes by expansion and gene acquisition. Nature 466:612–616

Bergero R, Forrest A, Kamau E, Charlesworth D (2007) Evolutionary strata on the X chromosomes of the dioecious plant *Silene latifolia*: evidence from new sex-linked genes. Genetics 175:1945–1954

Birchler JA, Veitia RA (2010) The gene balance hypothesis: implications for gene regulation, quantitative traits and evolution. New Phytol 186:54–62

Branciforte D, Martin SL (1994) Developmental and cell type specificity of LINE-1 expression in mouse testis: implications for transposition. Mol Cell Biol 14:2584–2592

Bull JJ (1983) Evolution of sex determining mechanisms. Benjamin Cummings, Menlo Park, California

Castagné R, Rotival M, Zeller T et al (2011) The choice of the filtering method in microarrays affects the inference regarding dosage compensation of the active X-chromosome. PLoS One 6:e23956

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

Charlesworth B, Charlesworth D (1978) A model for the evolution of dioecy and gynodioecy. Am Nat 112:975–997

Charlesworth D, Mank JE (2010) The birds and the bees and the flowers and the trees: lessons from genetic mapping of sex determination in plants and animals. Genetics 186:9–31

Chaumeil J, Waters PD, Koina E et al (2011) Evolution from XIST-independent to XIST-controlled X-chromosome inactivation: epigenetic modifications in distantly related mammals. PLoS One 6:1–11

Cohen DE, Davidow LS, Erwin JA, Xu N, Warshawsky D, Lee JT (2007) The DXPas34 repeat regulates random and imprinted X inactivation. Dev Cell 12:57–71

Davidow LS, Breen M, Duke SE et al (2007) The search for a marsupial XIC reveals a break with vertebrate synteny. Chromosome Res 15:137–146

Deakin JE, Hore TA, Koina E, Graves JAM (2008) The status of dosage compensation in the multiple X chromosomes of the platypus. PLoS Genet 4:1–13

Deakin JE, Chaumeil J, Hore TA, Graves JAM (2009) Unravelling the evolutionary origins of X chromosome inactivation in mammals: insights from marsupials and monotremes. Chromosome Res 17:671–685

Delbridge ML, Lingenfelter PA, Disteche CM, Graves JAM (1999) The candidate spermatogenesis gene RBMY has a homologue on the human X chromosome. Nat Genet 22:223–224

Delgado CLR, Waters PD, Gilbert C, Robinson TJ, Graves JAM (2009) Physical mapping of the elephant X chromosome: conservation of gene order over 105 million years. Chromosome Res 17:917–926

Ding W, Lin L, Cehn B, Dai J (2006) L1 elements, processed pseudogenes and retrogenes in mammalian genomes. IUBMB Life 58:677–685

Ellegren H, Hultin-Rosenberg L, Brunstrom B et al (2007) Faced with inequality: chicken do not have a general dosage compensation of sex-linked genes. BMC Biol 5:40–51

Engelstadter J, Haig D (2008) Sexual antagonism and the evolution of X chromosome inactivation. Evolution 62:2097–2104

Escamilla-Del-Arenal M, Teixeira da Rocha S, Heard E (2011) Evolutionary diversity and developmental regulation of X-chromosome inactivation. Hum Genet 130:307–327

Fisher RA (1931) The evolution of dominance. Biol Rev 6:345–368

Fraser JA, Diezmann S, Subaran RL et al (2004) Convergent evolution of chromosomal sex-determining regions in the animal and fungal kingdoms. PLoS Biol 2:2243–2255

Goddard MR, Godfray HC, Burt A (2005) Sex increases the efficacy of natural selection in experimental yeast populations. Nature 434:636–640

Goto H, Peng L, Makova KD (2009) Evolution of X-degenerate Y chromosome genes in greater apes: conservation of gene content in human and gorilla, but not chimpanzee. J Mol Evol 68:134–144

Gupta V, Parisi M, Sturgill D et al (2006) Global analysis of X-chromosome dosage compensation. J Biol 5:3

Haig D (2006) Self-imposed silence: parental antagonism and the evolution of X-chromosome inactivation. Evolution 60:440–447

Hense W, Baines JF, Parsch J (2007) X chromosome inactivation during *Drosophila* spermatogenesis. PLoS Biol 5:e273

Hore TA, Koina E, Wakefield MJ, Graves JAM (2007) The region homologous to the X-chromosome inactivation centre has been disrupted in marsupial and monotreme mammals. Chromosome Res 15:147–161

Hughes JF, Skaletsky H, Pyntikova T et al (2010) Chimpanzee and human Y chromosome are remarkably divergent in structure and gene content. Nature 463:536–539

Itoh Y, Melamed E, Yang X et al (2007) Dosage compensation is less effective in birds than in mammals. J Biol 6:2

Kaiser VB, Ellegren H (2006) Nonrandom distribution of genes with sex-biased expression in the chicken genome. Evolution 60:1945–1951

Kondo M, Nanda I, Hornung U, Schmid M, Schartl M (2004) Evolutionary origin of the medaka Y chromosome. Curr Biol 14:1664–1669

Kondo M, Hornung U, Nanda I et al (2006) Genomic organization of the sex-determining and adjacent regions of the sex chromosomes of medaka. Genome Res 16:815–826

Lahn BT, Page DC (1999) Four evolutionary strata on the human X chromosome. Science 286:964–967

Lemos B, Araripe LO, Hartl DL (2008) Polymorphic Y chromosomes harbor cryptic variation with manifold functional consequences. Science 319:91–93

Liu Z, Moore PH, Ma H et al (2004) A primitive Y chromosome in papaya marks incipient sex chromosome evolution. Nature 427:348–352

Lyon MF (1998) X-Chromosome inactivation: a repeat hypothesis. Cytogenet Genome Res 80:133–137

Lyon MF (2006) Do LINEs have a role in X-chromosome inactivation? J Biomed Biotechnol 2006:59746

Mahadevaiah SK, Royo H, VandeBerg JL et al (2009) Key features of the X inactivation process are conserved between marsupials and eutherians. Curr Biol 19:1478–1484

Mank JE (2009) The W, X, Y and Z of sex-chromosome dosage compensation. Trends Genet 25:226–233

Mank JE, Hosken DJ, Wedell N (2011) Some inconvenient truths about sex chromosome dosage compensation and the potential role of sexual conflict. Evolution 65:2133–2144

Marais G, Nicolas M, Bergero R et al (2008) Evidence for degeneration of the Y chromosome in the dioecious plant *Silene latifolia*. Curr Biol 18:545–549

Meiklejohn CD, Landeen EL, Cook JM, Kingan SB, Presgraves DC (2011) Sex chromosome-specific regulation in the *Drosophila* male germline but little evidence for chromosomal dosage compensation or meiotic inactivation. PLoS Biol 9:e1001126

Ming R, Bendahmane A, Renner SS (2011) Sex chromosomes in land plants. Annu Rev Plant Biol 62:485–514

Mueller JL, Mahadevaiah SK, Park PJ et al (2008) The mouse X chromosome is enriched for multicopy testis genes showing postmeiotic expression. Nat Genet 40:794–799

Murphy WJ, Wilkerson AJP, Raudsepp T et al (2006) Novel gene acquisition on carnivore Y chromosomes. PLoS Genet 2:353–363

Na J-K, Wang J, Murray JE et al. (2011) Rapid expansion of the hermaphrodite specific region of Y^h chromosome (HSY) in papaya (in press)

Nam K, Ellegren H (2008) The chicken (*Gallus gallus*) Z chromosome contains at least three nonlinear evolutionary strata. Genetics 180:1131–1136

Namekawa SH, VandeBerg JL, McCarrey JR, Lee JT (2007) Sex chromosome silencing in the marsupial male germ line. Proc Natl Acad Sci U S A 104:9730–9735

Naurin S, Hansson B, Bensch S, Hassequist D (2010) Why does dosage compensation differ between XY and ZW taxa? Trends Genet 26:15–20

Nguyen DK, Disteche CM (2006) Dosage compensation of the active X chromosome in mammals. Nat Genet 38:47–53

Ohno S (1967) Sex chromosomes and sex linked genes. In: Labhart A, Mann T, Samuels LT (eds) Monographs on endocrinology. Springer, Heidelberg

Okamoto I, Patrat C, Thepot D et al (2011) Eutherian mammals use diverse strategies to initiate X-chromosome inactivation during development. Nature 472:370–376

Oliver SG, de Clare M, Pir P (2011) Haploinsufficiency and the sex chromosomes from yeasts to humans. BMC Biol 9:15

Paland S, Lynch M (2006) Transitions to asexuality result in excess amino acid substitutions. Science 311:990–992

Peichel CL, Ross JA, Matson CK et al (2004) The master sex-determination locus in three spine sticklebacks is on a nascent Y chromosome. Curr Biol 14:1416–1424

Potrzebowski L, Vinckenbosch N, Marques AC, Chalmel F, Jégou B, Kaessmann H (2008) Chromosomal gene movements reflect the recent origin and biology of therian sex chromosomes. PLoS Biol 6:709–716

Potrzebowski L, Vinckenbosch N, Kaessmann H (2010) The emergence of new genes on the young therian X. Trends Genet 26:1–4

Ro S, Park C, Sanders KM, McCarrey JR, Yan W (2007) Cloning and expression profiling of testis-expressed microRNAs. Dev Biol 311:592–602

Ross JA, Peichel CL (2008) Molecular cytogenetic evidence of rearrangements on the Y chromosome of threespine stickleback fish. Genetics 179:2173–2182

Ross MT, Grafham DV, Coffey AJ et al (2005) The DNA sequence of the human X chromosome. Nature 434:325–337

Siroky J, Castiglione MR, Vyskot B (1998) DNA methylation patterns of *Melandrium album* chromosomes. Chromosome Res 6:441–446

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

Slotkin RK, Martienssen R (2007) Transposable elements and the epigenetic regulation of the genome. Nat Rev Genet 8:272–285

Song R, Ro S, Michaels JD, Park C, McCarrey JR, Yan W (2009) Many X-linked microRNAs escape meiotic sex chromosome inactivation. Nat Genet 41:488–493

Spigler RB, Lewers KS, Main DS, Ashman TL (2008) Genetic mapping of sex determination in a wild strawberry, *Fragaria virginiana*, reveals earliest form of sex chromosome. Heredity 101:507–517

Springer MS, Murphy WJ, Eizirik E, O'Brien SJ (2003) Placental mammal diversification and the Cretaceous-Tertiary boundary. PNAS 100:1056–1061

Storchova R, Divina P (2006) Nonrandom representation of sex-biased genes on chicken Z chromosome. J Mol Evol 63:676–681

Tsuda Y, Nishida-Umehara C, Ishijima J, Yamada K, Matsuda Y (2007) Comparison of the Z and W sex chromosomal architectures in elegant crested tinamou (*Eudromia elegans*) and ostrich (*Struthio camelus*) and the process of sex chromosome differentiation in palaeognathous birds. Chromosoma 116:159–173

Tuskan GA, DiFazio S, Jansson S et al (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. and Gray). Science 313:1596–1604

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

Vibranovski MD, Lopes HF, Karr TL, Long M (2009) Stage-specific expression profiling of *Drosophila* spermatogenesis suggests that meiotic sex chromosome inactivation drives genomic relocation of testis-expressed genes. PLoS Genet 5:1–13

Vicoso B, Bachtrog D (2009) Progress and prospects toward our understanding of the evolution of dosage compensation. Chromosome Res 17:585–602

Vicoso B, Bachtrog D (2011) Lack of global dosage compensation in *Schistosoma mansoni*, a female-heterogametic parasite. Genome Biol Evol 3:230–235

Warburton PE, Giordano J, Cheung F, Gelfand Y, Benson G (2004) Inverted repeat structure of the human genome: the X-chromosome contains a preponderance of large, highly homologous inverted repeats that contain testes genes. Genome Res 14:1861–1869

Weismann A (1889) The significance of sexual reproduction in the theory of natural selection. In: Poulton EB, Schönland S, Shipley AE (eds) Essays upon heredity and kindred biological problems. UK Clarendon, Oxford, pp 261–305

Wolf JBW, Bryk J (2011) General lack of global dosage compensation in ZZ/ZW systems? Broadening the perspective with RNA-seq. BMC Genomics 12:91–100

Xiong YY, Chen XS, Chen ZD et al (2010) RNA sequencing shows no dosage compensation of the active X-chromosome. Nat Genet 42:1043–1047

Yamato KT, Ishizaki K, Fujisawa M et al (2007) Gene organization of the liverwort Y chromosome reveals distinct sex chromosome evolution in a haploid system. Proc Natl Acad Sci U S A 104:6472–6477

Yin T, DiFazio SP, Gunter LE et al (2008) Genome structure and emerging evidence of an incipient sex chromosome in Populus. Genome Res 18:422–430

Yu Q, Hou S, Feltus FA et al (2008) Low X/Y divergence in four pairs of papaya sex-liked genes. Plant J 53:124–132

Yu Q, Tong E, Skelton RL et al (2009) A physical map of the papaya genome with integrated genetic map and genome sequence. BMC Genomics 10:371

Zha X, Xia Q, Duan J et al (2009) Dosage analysis of Z chromosome genes using microarray in silkworm, *Bombyx mori*. Insect Biochem Mol Biol 39:315–321

Zhang W, Wai CM, Ming R, Yu Q, Jiang J (2010a) Integration of genetic and cytological maps and development of a pachytene chromosome-based karyotype in papaya. Trop Plant Biol 3:166–170

Zhang YE, Vibranovski MD, Landback P, Marais GAB, Long M (2010b) Chromosomal redistribution of male-biased genes in mammalian evolution with two bursts of gene gain on the X chromosome. PLoS Biol 8:1–13