Sex-chromosome evolution: recent progress and the influence of male and female heterogamety

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Abstract | It is now clear that sex chromosomes differ from autosomes in many aspects of genome biology, such as organization, gene content and gene expression. Moreover, sex linkage has numerous evolutionary genetic implications. Here, I provide a coherent overview of sex-chromosome evolution and function based on recent data. Heteromorphic sex chromosomes are almost as widespread across the animal and plant kingdoms as sexual reproduction itself and an accumulating body of genetic data reveals interesting similarities, as well as dissimilarities, between organisms with XY or ZW sex-determination systems. Therefore, I discuss how patterns and processes associated with sex linkage in male- and female-heterogametic systems offer a useful contrast in the study of sex-chromosome evolution.

Reproduction by means of sex is a common theme in the transmission of genetic information between generations in multicellular organisms. Sexual reproduction includes the fusion of gametes from two individuals during fertilization, leading to the formation of a zygote. The gametes are either similar (isogamy) or dissimilar (anisogamy) in size and form, and it is anisogamy that can give rise to the existence of males and females as separate sexes[1,2]. Sex can be determined by the environment or be genetically controlled by one or more loci located on either an autosome or on sex chromosomes[3,4].

Sex chromosomes have evolved independently numerous times in different lineages, yet they show many common features and thus represent a fascinating example of evolutionary convergence. The process of sex-chromosome evolution has attracted considerable interest over the years, and an important question has been what the evolutionary forces are that act to make a pair of autosomes cease recombining in one sex, eventually leading to the formation of two discrete chromosome types. Other questions have surrounded the genomic organization of sex chromosomes and the evolutionary implications of sex linkage. Do the gene content and other genomic characteristics differ between autosomes and sex chromosomes and, if so, why[5]? Do sex-linked genes evolve differently compared with autosomal genes and, if so, which evolutionary forces can explain such a difference[6]? Furthermore, are sex chromosomes hotspots for loci relating to adaptation and speciation[7]? Although some questions have been backed up by a framework of theoretical expectations, these and other questions relating to sex-chromosome evolution have, until recently, been difficult to address in detail owing to a lack of empirical data. However, during the past few years, access to large-scale sequence and expression data have led to new insight into sex-chromosome evolution.

In this Review, I discuss the most recent advances in the genetics of sex chromosomes, including sex-chromosome evolution, sex-chromosome organization and the molecular evolution of sex-linked genes. Most of our knowledge on the genetics and genomics of sex chromosomes, and the evolutionary implications of sex linkage, has come from studies of organisms with male heterogamety (males XY, females XX) — for example, mammals and *Drosophila*. An accumulating body of data from organisms with female heterogamety (males ZZ, females ZW; BOX 1) is now revealing interesting similarities, as well as dissimilarities, between organisms with XY or ZW sex-determination systems. Comparisons of patterns and processes associated with sex linkage in XY and ZW systems offer a useful contrast because they can disentangle effects caused by sex-chromosome organization from those caused by sex *per se*. For example, whereas sexual selection, which occurs predominantly among males, has the potential to directly influence evolution of the sex-limited (male-specific) chromosome in male heterogametic organisms, this is not the case for the sex-limited (female-specific)

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chromosome in organisms with female heterogamety. Female heterogametic organisms thus constitute a natural laboratory in which to independently test hypotheses concerning sex-chromosome evolution and sex linkage that have been developed, inspired by work in male heterogametic systems.

Sex-chromosome systems

Sex chromosomes are labile. It is clear that sex chromosomes constitute a genetic system characterized by an unusually dynamic evolution, in several respects. Not only are there male and female heterogamety, but some groups of species — such as teleost fishes[8] and amphibians[9] — have both sex-chromosome systems represented even without a clear phylogenetic signal in the distribution of XY versus ZW systems over the group's tree of species. An extreme example of the evolutionary instability of sex chromosomes is seen in the frog *Rana rugosa*, in which XY and ZW systems co-occur within the same species, being fixed in different populations[10]. Comparative mapping has shown that sex-chromosome evolution can occur truly independently because sex chromosomes from related lineages are not necessarily homologous[11–13]. Indeed, recent theoretical work suggests that the evolutionary lability of genetic sex determination can be explained by the spread of new sex-determining loci linked to autosomal loci under sexually antagonistic selection[14], which can in turn trigger transitions between male and female heterogamety[15].

There is considerable variation on a standard theme of an XY/XX or a ZW/ZZ design. For example, some organisms have only one type of sex chromosome — that is, X0/XX or Z0/ZZ systems. In other cases, there are recently evolved sex chromosomes (neo-sex chromosomes) formed by fusions or translocations involving autosomes, giving systems such as Z_1Z_2W/$Z_1Z_1Z_2Z_2$ and XY_1Y_2/XX, to name just two examples. Some plants, invertebrates and, as recently discovered, the platypus and echidnas (the monotreme mammals) have a spectacular sex-chromosome system consisting of meiotic multiples of sex chromosomes that form rings or chains[16]. In monotremes, this sex-chromosome chain has the organization $X_1X_2X_3X_4X_5$/$Y_1Y_2Y_3Y_4Y_5$ in male meiosis. One conclusion that can be drawn from this is that the 'simple' organization of XY males and XX females — which is seen in essentially all eutherian mammals and may sometimes be taken as the standard sex-chromosome design — clearly does not reflect the variety of sex-chromosome systems and the rapid transitions that occur during sex-chromosome evolution in many evolutionary lineages.

Sex determination. The two principal ways in which sex chromosomes can be involved in sex determination are by the sex-limited chromosome taking a dominant role (for example, in mammals) or by means of a counting mechanism in which the dose of the X (or Z) chromosome relative to the autosomes determines sex (for example in *Drosophila* and *Caenorhabditis elegans*). Recently, the basis for sex determination in various other species, including fishes, insects, plants and chickens, has been revealed[17]. This work corroborates early observations[18] that the upstream triggers of the cascade of genes involved in sex determination often vary among organisms, whereas downstream components of the cascade tend to be conserved. An example of a conserved downstream component is the human gene double-sex mab-3 related transcription factor 1 (*DMRT1*), which contains a highly conserved DNA-binding motif that is also present in the *Drosophila melanogaster doublesex* and the *C. elegans mab-3* sexual regulatory genes[19]. In humans, the gene's crucial role in male development is indicated by the finding that monoallelic (hemizygotic) expression of *DMRT1*, located on chromosome 9, is associated with haploinsufficiency in the form of XY sex reversal[20]. This suggests that a double gene dose is necessary for normal sexual development in males.

Interestingly, a similar situation seems to occur in birds. The mechanism of sex determination in birds has long been an enigma. Is it the presence of the female-specific W chromosome that triggers female development through a dominant ovary-determining gene (a male phenotype being the default form) or is there a counting mechanism, with the number of Z chromosomes relative to the number of autosomes determining sex? *DMRT1* is Z-linked in all birds, and by using RNA interference to knock down DMRT1 expression, researchers have recently shown that reduced expression in genetically male (ZZ) chicken embryos leads to feminization of the developing gonads and partial sex reversal[21]. As in mammals, a double dose of DMRT1 thus seems necessary for normal male development. This does not strictly prove that *DMRT1* is the master determinant of sex in birds because there could still be

Box 1 | The occurrence of female heterogamety

Male heterogamety is the most common sex-chromosome system found across the animal kingdom, as well as the type seen in dioecious plants in which sex chromosomes occur. As in animals, plant sex chromosomes have evolved independently several times, for example, in angiosperms[108]. Female heterogamety is found in a diverse range of organisms, including birds, reptiles, amphibians, fishes, crustaceans, and moths and butterflies. Birds, moths and butterflies seem to be the only groups of species in which female heterogamety is the norm (although as far as moths and butterflies are concerned, only a small minority of the more than 130,000 lepidopteran species known have so far been investigated). In the other groups mentioned, female heterogamety is present in some lineages and male heterogamety in others, with some lineages even showing environmental sex determination.

In birds, comparative cytogenetic work suggests that the Z and W chromosomes are largely homologous in all species[109] and molecular dating indicates that Z and W started to differentiate before the most basal split of extant lineages, at least 140 million–120 million years ago[29]. Birds belong to the group of saurischian dinosaurs and it is not known whether their closest non-avian dinosaur relatives, such as tyrannosaurs, oviraptors and velociraptors, also had female heterogamety (or even sex chromosomes). The avian sex chromosomes show homology with the Z and W chromosomes of some geckos[110], although not with other lizards that have female heterogamety[111]; the gecko–bird sex-chromosome homology may, therefore, represent a case of convergence rather than of shared ancestry. In Lepidoptera, data from a limited number of species suggest that the Z chromosome has retained conserved synteny across the whole group. Moreover, female heterogamety is also found in caddis flies (Trichoptera), the sister group to moths and butterflies, suggesting that their sex chromosomes had already started to evolve in a common ancestor of Lepidoptera and Trichoptera more than 190 million years ago[33].

other genes acting upstream, including Z-linked and/or W-linked genes. However, it demonstrates a great deal of similarity between the sex-determining pathways in two vertebrate lineages, even though one has male and the other female heterogamety. Notably, birds lack chromosome-wide full-dosage compensation of Z-linked genes[22,23], so there is potential for sex-specific processes associated with Z-chromosome dose.

Sex-chromosome evolution

A consensus model for sex-chromosome evolution has been developed that posits that sex chromosomes originate from an ordinary pair of autosomes harbouring a sex-determining locus between which recombination becomes suppressed[24]. One way in which this could come about is through inversions (or other chromosomal rearrangements) that include the sex-determining locus and impose a direct hindrance to crossing over between the proto-sex chromosomes during meiosis. Another possibility is that natural selection acts on a modifier locus to favour cessation of recombination between the proto-sex chromosomes. Consider a sex-determining locus on an autosome where one allele triggers the development of, for example, a male phenotype. If there is another locus on the same chromosome that contains an allele that confers a selective advantage to males, but is neutral or even disadvantageous to females (sexual antagonism), then selection should favour suppressed recombination when the male-determining allele and the male-advantageous allele are in phase on the same chromosomal copy. Selection against recombination would thus occur only in males and, as a consequence, the non-recombining chromosome would become male-specific and males the heterogametic sex. In females, the proto-sex chromosomes would continue to recombine. In principle, the same scenario is possible in a situation of a female-determining allele and a female-advantageous allele at a sexually antagonistic locus located on the same chromosome, in this case leading to female heterogamety.

A model of sex-chromosome evolution including suppressed recombination between an ancestral pair of autosomes implies that the non-recombining and the still recombining chromosome share a common ancestry. Over time and in the absence of recombination, the two chromosomes will independently accumulate mutations that make them increasingly different from each other. Quantification of these differences in paralogous (gametologous[25]) sequences shared between the sex chromosomes should, in principle, provide a means of dating the start of their independent evolution. Lahn and Page[26] used this approach and found that the degree of divergence between the human X and Y chromosomes varies considerably among loci. When noting the physical arrangement of these loci on the X chromosome, they found more or less discrete clusters with respect to the degree of divergence, suggesting a historical series of progressive reductions in recombination (FIG. 1a). These clusters, termed 'evolutionary strata', are ordered so that the cluster with the lowest degree of divergence between X–Y paralogues is located next to the pseudoautosomal region (PAR) on Xp, with the other three clusters showing increasing divergence with increasing distance from PAR. A fifth stratum has subsequently been suggested[27]. By applying a molecular clock to the observed divergence within each cluster, it was estimated that the most recent stratum was formed 50 million–30 million years ago[26] (FIG. 1b). Stepwise cessation of recombination between the human X and Y chromosomes thus seems to have been initiated before mammalian radiation, with the last event occurring specifically in the primate lineage. Evolutionary strata on sex chromosomes have now been seen in a wide range of organisms, including plants[28] and birds[29], as well as in non-recombining regions of the mating-type chromosome in fungi[30,31]. The formation of evolutionary strata on sex chromosomes is compatible both with a model of selection against recombination associated with sexually antagonistic loci and with inversion events[32]. Notably, strata occur both in XY and ZW systems, highlighting a common mechanism of sex-chromosome evolution irrespective of type of heterogamety.

Sex-chromosome evolution does not always follow the processes described above. For example, in Lepidoptera, it has been suggested that the ancestral sex-chromosome system was Z0/ZZ and that the W chromosome arose at a later stage[33], an interpretation made on the basis of the fact that the most basal clades lack a W chromosome. However, in theory, the W chromosome could have been lost from these clades. Moreover, there are numerous examples of *de novo* formation of Y chromosomes in male heterogametic systems by fusion or translocation events involving autosomes[34]. Finally, it seems to be an overlooked problem that the model of sex-chromosome evolution by selection for reduced recombination between a dominant allele at a sex-determining locus and sexually antagonistic genes on the same chromosome is less easily conceived in a situation whereby sex is determined by sex-chromosome dosage.

Sex-chromosome organization

The sex-limited chromosome. As will be discussed in the next section, the absence of recombination in the sex-limited chromosome makes it vulnerable to deleterious mutations, eventually leading to pseudogenization and gene loss. Y (or W) chromosomes, as observed in model systems with old sex chromosomes, have lost most of the several hundred genes that were once present in their recombining proto-sex chromosomes. They are usually rich in heterochromatin, filled with satellite DNA-like repeats and contain only a limited number of genes. In the mammalian Y chromosome most of these genes have testis-specific expression. Extensive sequencing efforts for the human Y chromosome have revealed that it is filled with ampliconic and palindromic structures, including multiple copies of a handful of genes[35] (FIG. 2). In fact, the great majority of genes on the human Y chromosome belong to nine families. In the palindromic regions there is extensive structural variation among individuals, which indicates that these regions are unstable[36]. Furthermore,

Sexual antagonism
Sexually antagonistic genes are genes for which expression has contrasting effects on fitness in the two sexes.

Gametologous
Gametologous genes are homologous genes shared between the X and Y, or Z and W, chromosomes that have evolved independently since recombination ceased in the ancestral gene.

Pseudoautosomal region
(PAR). The region of both sex chromosomes that still recombines in the heterogametic sex. In old and highly differentiated sex chromosomes, such as the mammalian X and Y, the PAR is usually small.

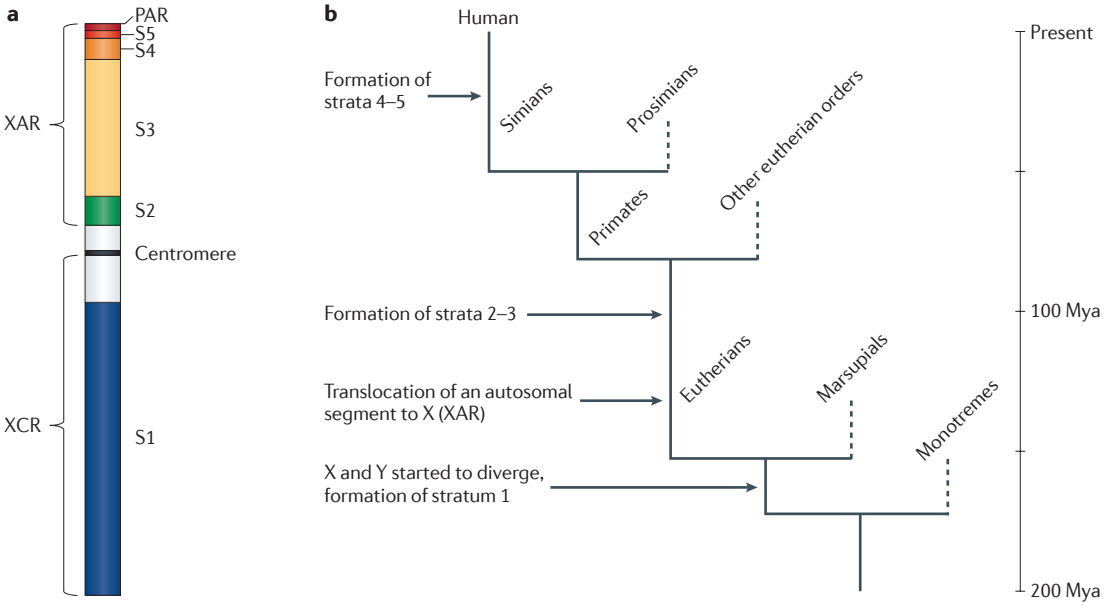

Figure 1 | **Organization of the human X chromosome. a** | A schematic illustration of the human X chromosome. The evolutionary history of this chromosome includes a region defined as the X-conserved region (XCR) that is present on the X chromosome of marsupials but is autosomal in monotremes. This indicates that in therians (placental mammals plus marsupials) sex chromosomes started to evolve sometime between therians' split from monotremes (about 170 million years ago) and the split between eutherians (placental mammals) and marsupials (about 150 million years ago). The X-added region (XAR) is of autosomal origin and is considered to have been added to the human X chromosome after the split between eutherians and marsupials but before the radiation of eutherians (150 million–100 million years ago)[118]. The pseudoautosomal region (PAR) recombines with a corresponding region on the Y chromosome during male meiosis. S1–S5 denote the five evolutionary strata that have been identified[26,27]. Each stratum tentatively represents the result of stepwise cessation of recombination between the proto-sex chromosomes. **b** | A tree showing the approximate timing of events associated with the evolution of the human X chromosome. Mya, million years ago.

gene copies frequently get homogenized by interallelic Y-to-Y gene conversion[37,38], a recombination-related process that apparently occurs even though the male-specific region of the Y chromosome does not recombine at meiosis. This can be an efficient, and perhaps the only, way to retard degeneration of gene function in the face of asexual transmission of the Y chromosome[39,40]. It is therefore possible that gene amplification on the Y chromosome is an adaptive trait that has been favoured by natural selection during evolution. In addition, there are examples of how X-to-Y conversion can counteract degeneration of Y-linked genes[41,42].

The lability of Y-chromosome structures is further indicated by interspecific comparisons. The recent sequencing of the chimpanzee Y chromosome revealed that, as far as this chromosome is concerned, humans and chimpanzees differ radically in sequence structure and gene content (FIG. 2), indicating rapid evolution during the past 6 million years[43]. These differences include expansions of palindromic structures and loss of large fractions of protein-coding genes. Similar observations have been made for the *Drosophila* Y chromosome in interspecific comparisons[44]. As yet, no W chromosome of a female-heterogametic organism has been sequenced at sufficient resolution to address structural organization. However, the avian W chromosome contains at least one ampliconic gene family, *HINTW*, in which frequent gene conversion occurs among copies[45].

Gene conversion
The transfer of genetic material from one chromosomal region to another. The 'donor' locus remains intact whereas the 'acceptor' locus changes. Gene conversion occurs between more or less homologous sequences.

Sex chromosomes found in both sexes. Is the X (or Z) chromosome organized similarly to an autosome? Given that it originated from an autosomal progenitor, one might think that this should be the case, unless the particular pair of autosomes from which the sex chromosomes evolved had specific properties. One might also think that the X (or Z) chromosome, in contrast to the Y (or W) chromosome, should have remained relatively unaffected in terms of organization and gene content during the course of sex-chromosome evolution. However, there are several lines of recent evidence that indicate the contrary. For example, long interspersed repeat elements are enriched on both the mammalian X and the avian Z chromosome[46,47], whereas gene density is lower than on autosomes in both systems as a result of intergenic expansions[27,48]. Moreover, three types of observation demonstrate that the type of gene in the X (or Z) chromosome is not representative of the genome as a whole.

First, genes with sex-biased expression are under- or over-represented on the X (or Z) chromosome in essentially all organisms that have been investigated[49]. Different levels of gene expression in the two sexes could be a consequence of the resolution of sexual antagonism (but see alternative evidence in REF. 50). By altering the expression level in the sex that suffers a selective disadvantage from the phenotype encoded by that gene, the sex-averaged fitness would increase. The

fact that the two sexes differ in terms of their chromosomal complement has implications for the evolution of sexually antagonistic genes and their genomic distribution[49]. In general, such genes are expected to evolve when the benefits to one sex outweigh the disadvantages incurred by the other[51]. However, when sexually antagonistic genes are sex linked, other scenarios are possible[49,52]. Because the X (or Z) chromosome spends more time in one sex than in the other, selection on a sex-linked allele will occur more frequently when it is in the homogametic sex than in the heterogametic sex. For dominant mutations, a sex-linked allele beneficial to the homogametic sex may thus go to fixation even if the absolute value of the (negative) selection coefficient in the heterogametic sex is higher than the (positive) coefficient for the homogametic sex. On the other hand, for recessive mutations a new allele beneficial to the heterogametic sex but deleterious to the homogametic sex will have a head start when sex-linked because it will be directly selected for in the heterogametic sex. In the homogametic sex it will not be selected against until it has reached sufficient frequency to start appearing in homozygote form.

Microarray data provide evidence for de-masculinization of the *Drosophila* spp. X chromosome in the form of a depletion of genes with male-biased expression[53–55]. This is consistent with a model of at least partial dominance of mutations underlying the evolution of sex-biased gene expression. A deficit of male-biased genes on the X chromosome could also relate to the process of meiotic sex-chromosome inactivation (MSCI)[56]. During this process, many genes on the X chromosome become temporarily silenced late in spermatogenesis; an autosomal location of genes important to the development of male germ cells should therefore be selectively favoured[57]. Support for this interpretation comes from stage-specific expression profiling of *Drosophila* male germ cells in which reduced expression of X-linked genes is confined to the time at which MSCI occurs[58–61], however, conflicting data have been reported by other researchers[53].

The situation might be more complex in mammals. Female-biased genes expressed in the ovaries and placenta are over-represented on the mouse X chromosome[62], as expected for partially dominant mutations. However, it has been suggested that male-biased genes expressed in the testes are also in excess on the mouse X chromosome[63,64]. Again, the precise timing in relation to MSCI could be important because there were indications that the excess was present only at the earliest stage of spermatogenesis, when germ cells are still diploid and MSCI has not set in, whereas at later stages, during meiosis, male-biased genes were under-represented on the X chromosome[62]. This suggested that a complex suite of evolutionary forces affects the distribution of sex-biased genes in the mammalian genome. Importantly however, more recent, better-resolved data have revealed that there is no overall excess of X-linked male-biased genes in the mouse testes. The claimed excess is seen only for a small proportion of recently evolved genes, whereas the more abundant category of old genes does not show this pattern[65].

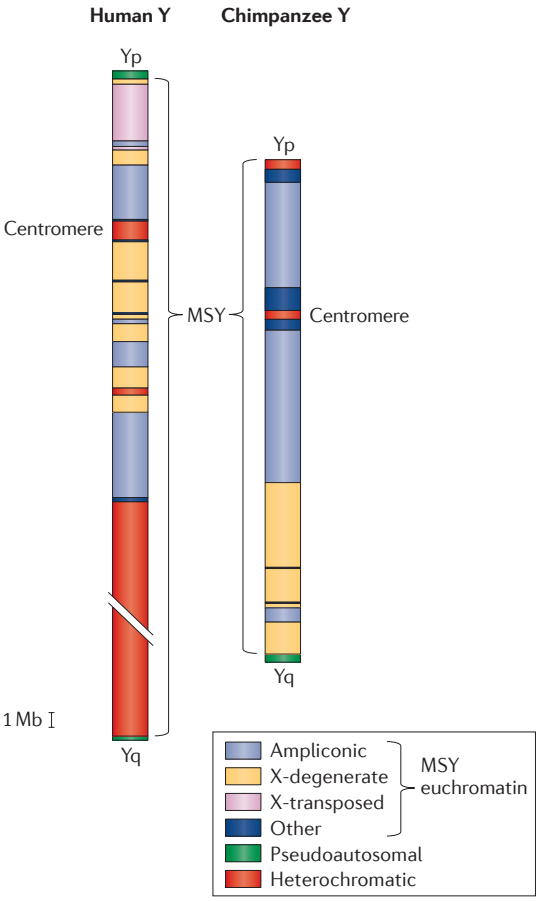

Figure 2 | **Organization of human and chimpanzee Y chromosomes.** The human and chimpanzee Y chromosomes differ considerably in organization and structure despite the recent divergence of the two lineages (about 6 million years ago). Ampliconic regions contain massive palindromic arrays of multi-copy testis-specific genes. The X-degenerate regions denote segments homologous to the X chromosome that were present on the proto-sex chromosomes before they started to diverge into X and Y. The X-transposed region on the human Y chromosome is a segment transposed from the X chromosome to the Y chromosome in the human lineage after the split from chimpanzee[119]. Most parts of the human and chimpanzee Y chromosomes do not recombine during male meiosis and are referred to as the male-specific region on Y (MSY). The figure is modified, with permission, from REF. 43 © (2010) Macmillan Publishing Limited. All rights reserved.

Recent studies of the genomic distribution of sex-biased genes in female heterogametic systems have provided interesting parallels. There is a deficit of female-biased genes expressed in the germ line of the chicken Z chromosome[66–69]. However, genes expressed in somatic granulosa cells are over-represented on the Z chromosome, although this is not the case for genes expressed in primary oocytes[69]. It should be noted that MSCI has recently been documented in chicken oogenesis[70], which may explain the above observation. In silkworms, testis-specific genes are enriched on the

Granulosa cells
Somatic cells in the ovary that surround the oocyte.

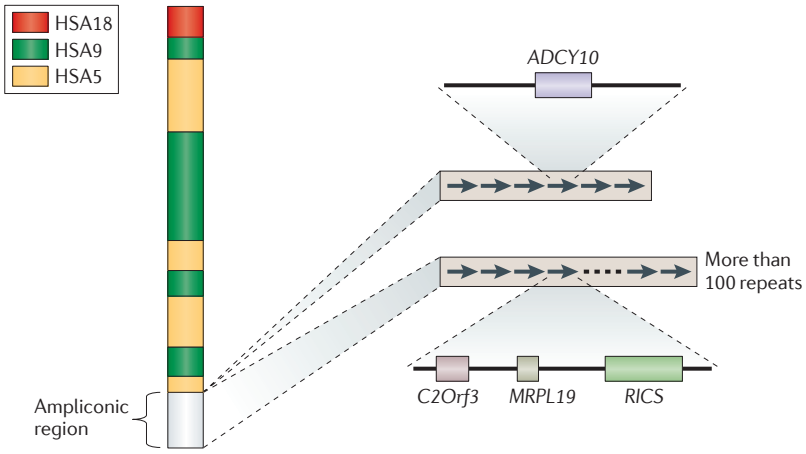

Figure 3 | **Organization of the chicken Z chromosome.** Coloured segments depict the homology between the chicken Z chromosome and different autosomes in the human genome (*Homo sapiens* autosome 5 (HSA5), HSA9 and HSA18). The ampliconic 5 region on the chicken Z chromosome contains a small array that includes six copies of the testis-specific gene adenylate cyclase 10 (*ADCY10*) and a massive array containing more than 100 copies of three testis-specific genes, *C2Orf3*, mitochondrial ribosomal protein L19 (*MRPL19*) and Rho GTPase activating protein 32 (*RICS*). Data from REF. 48.

Z chromosome[71] — another observation that is consistent with the accumulation of sex-linked, partly dominant mutations beneficial to the homogametic sex.

Second, when copies of genes are spread around the genome it is possible that their origin, as well as integration, is non-random with respect to chromosomal location. The first indication of this phenomenon came from the observation of extensive traffic of functional retrotransposed gene copies in and out of the mammalian[72–74] and the *Drosophila*[75,76] X chromosome. Among the genes that generate new retrocopies, through mRNA intermediates, there is an excess of X-linked genes inserted at autosomal locations. This process may be selectively driven in relation to the escape from MSCI, as discussed in the previous paragraph. A dominant male-beneficial allele that confers a cost to females will spread more easily through a population if it is autosomal than if it is X-linked. Consistent with this idea, retrotransposed gene copies leaving the X chromosome often evolve male-specific functions[72]. Interestingly, the X chromosome is also a hotspot for accepting new, functional retrocopies. Here, selection could potentially also be invoked, in this case under the assumption that male-beneficial genes are recessive and directly exposed to selection in hemizygotic males when X-linked. There is now evidence for similar non-random gene movements through DNA-based gene duplication or translocation in mammals and *Drosophila*[65,77,78].

Third, it has recently been recognized that the mammalian X[27] — as well as the avian Z[48] — chromosome harbours ampliconic gene structures similar to those seen on the Y and W chromosomes. The evolutionary history of these X- or Z-linked structures seems to follow a common theme: acquisition, evolution of male-specific function and amplification. In sequencing the chicken Z chromosome to near completion, Bellott *et al.*[48] identified four gene families, each with hundreds of gene copies, located within a massive 11-Mb tandem repetitive array representing 15% of the chromosome. These genes, which are single-copy and autosomal in outgroup species, have been relocated to the Z chromosome during avian evolution, are specifically expressed in testis tissue and have been subject to repeated rounds of duplication (FIG. 3).

All these observations challenge the standard model of sex-chromosome evolution in which most change was thought to occur in the chromosomes found in only one sex (the Y and W chromosomes), whereas the sex chromosomes found in both sexes (the X and Z chromosomes) were assumed to have diverged little from their autosomal progenitors. The available evidence now indicates that X and Z have an unusual complement of genes, with a non-random occurrence of genes involved in sex-specific, reproductive functions. This makes an interesting connection to the role of sex-linked genes in speciation, as outlined in BOX 2.

Molecular evolution

Mutation rates. The evolution of sex-linked DNA sequences is, in principle, governed by the same forces as in autosomal DNA, including mutation, selection and recombination. However, given the special mode of inheritance of sex chromosomes, the effect of these evolutionary forces differs between sex chromosomes and autosomes. In fact, a useful way to study the roles of different evolutionary forces on the evolution of DNA has been to contrast patterns of molecular evolution in the two chromosomal categories.

Mutation rates vary on several genomic scales, from sequence-context effects on the rate at individual nucleotides to variation among chromosomes. In addition to such intrinsic variation in mutability, mutation rates are also affected by the age and sex of the individual in which the mutations occur. It is often assumed that a substantial proportion (although not necessarily all[79]) of germline mutations results from replication errors during mitosis. If the per-cell generation mutation rate in the germ line is relatively constant, it follows that there should be a positive correlation between the number of germline cell divisions and the number of mutations that eventually accumulate in gametes. Whereas cell division in human oogenesis is arrested at an embryonic stage with a finite number of mitotic cell divisions, in spermatogenesis germ cells continue to divide at a high rate during adulthood to ensure lasting sperm production. Because of this, the mutation rate should be higher in males than in females, and higher in older than in younger males, and this has been observed[80].

In male-heterogametic systems, the Y chromosome is transmitted only from fathers to sons and will therefore be affected only by the male mutation rate. The X chromosome is transmitted through the male germ line one-third of the time and through the female germ line two-thirds of the time, being affected by male and female mutation rates accordingly. As a consequence, the Y chromosome should have a higher mutation rate than the X chromosome[81]. Taking divergence at neutral sites as a proxy for the rate of mutation, this has been observed in many organisms[82–84]. In humans, Y-chromosomal divergence is

Box 2 | **Sex-linked genes and their role in speciation**

The non-random nature of the gene content of sex chromosomes offers an intriguing link to the role of sex chromosomes in speciation. It has been recognized that there is a particularly large effect of X-linked loci on postmating reproductive isolation — a key aspect of speciation — as manifested in hybrid sterility[112,113]. This 'large X effect' can be explained in several ways[7], including by invoking trivial methodological artefacts associated with the increased possibility of detecting X-linked recessive speciation genes in backcross analysis. However, there are now data from genome-wide introgression analysis to suggest that hybrid sterility factors are overrepresented on at least the *Drosophila* X chromosome[114]. So why is this?

One interesting hypothesis focuses on meiotic sex-chromosome inactivation (MSCI) in the male germ line. If the MSCI machinery is unable to recognize and silence introgressed heterospecific segments on the X chromosome, this may lead to overexpression of X-linked genes that impair spermatogenesis[114,115]. There is empirical evidence from hybrid mice that failure of MSCI in late stages of spermatogenesis leads to X-chromosome-wide disruption of gene expression[116]. An associated possibility is related to the imbalance between the X chromosome and autosomes in male hybrids, originally proposed to explain Haldane's rule. Whereas female hybrids have one complete haploid genome from each of the two parental species, male hybrids may suffer from the loss of epistatic interactions between the absent parental X chromosome and the corresponding autosomal alleles. Recently, Lu *et al.*[117] have demonstrated that the X-linked *Drosophila* speciation gene *Odysseus* causes misexpression of numerous autosomal genes involved in spermatogenesis in male hybrids, consistent with the observation that the large X effect primarily manifests in hybrid sterility rather than hybrid inviability.

about twofold higher than X-chromosomal divergence, corresponding to a male-to-female mutation rate ratio of between 5 and 6. In essence, the high mutation rate on the Y chromosome increases the risk of deleterious mutations entering the population. In female-heterogametic systems, the analogous argument implies that the Z chromosome (transmitted through the male germ line two-thirds of the time) is expected to have a higher mutation rate than the W chromosome (transmitted only from mothers to daughters), which is observed[85]. Because of the specific mutation pressure acting on sex chromosomes, attempts to derive null models of levels of sex-linked genetic diversity need to take the difference in mutation load between X (or Z) and Y (or W) into account.

Degeneration and diversity of the sex-limited chromosome. The effective population size (N_e) of the Y (or W) chromosome is considerably smaller than that of either the X (or Z) chromosome (by one-third) or the autosomes (by one-quarter). Selection may act to augment these differences. Recombination ensures that selection can operate more or less independently at different loci. By contrast, in a non-recombining chromosome, selection at one locus will also affect other loci, thereby reducing N_e of the non-recombining region. In general, reduced N_e implies that a population can maintain lower levels of genetic diversity and that deleterious mutations have a higher chance of reaching fixation by genetic drift. Together, these situations are expected to lead to the degeneration of the sex-limited chromosome. As deleterious mutations become fixed in protein-coding genes on the Y (or W) chromosome, they may eventually turn into pseudogenes. At this point, deletions or structural rearrangements involving these genes will not be selected against, leading to gradual loss of DNA from the Y (or W) chromosome.

The processes thought to reduce the N_e of Y (or W) chromosomes have been discussed in detail elsewhere[86] and include Muller's ratchet, Hill–Robertson interference, selective sweeps and background selection. Their relative importance is far from understood and may well vary among organisms. Empirical data on allele frequency spectra in the neo-Y chromosome of *Drosophila miranda* was taken as support for the degeneration of Y-linked genes through recent positive selection (selective sweeps)[87]. Subsequent work has suggested that Muller's ratchet can explain many of the loss-of-function mutations in the *D. miranda* Y chromosome[88,89]. Moreover, there is probably a temporal component, with Muller's ratchet and background selection dominating in newly formed, non-recombining chromosomes, when there still are many active gene targets at which deleterious mutation can occur (assuming additivity)[90,91].

Sexual selection usually implies that some males father a disproportionate share of the offspring (polygyny). In male heterogametic systems this further reduces the N_e of Y chromosomes relative to that of X chromosomes and autosomes. Moreover, the fact that many Y-linked genes are involved in male reproduction may set the stage for selective sweeps related to sperm competition, a key component of sexual selection. Does this suggest that Y chromosomes are more prone to degeneration than W chromosomes in systems of female heterogamety? Simulations would be needed to address this quantitatively, however, W chromosomes have several features that closely resemble those of Y chromosomes. W chromosomes are typically small and filled with repetitive heterochromatin, have lost most of the genes once present in the recombining proto-sex chromosomes[92] and the remaining W-linked genes show an elevated rate of non-synonymous substitution compared with their Z-linked paralogues[93].

The prediction of lowered levels of genetic diversity on the sex-limited chromosome resulting from low N_e has empirical support from several organisms[94–96]. In humans, Y-chromosomal nucleotide diversity has been estimated to be one nucleotide difference every 10 kb in pairwise comparisons of unrelated Y chromosomes[97,98]. This is about ten times lower than is typically seen in autosomal human DNA. Similar observations have been made for the W chromosome in female-heterogametic systems[99], which suggests that the evolutionary forces that result in reduced diversity in the sex-limited chromosome are relatively independent of the type of heterogamety.

Molecular evolution of the X (or Z) chromosome. Stimulated by theoretical arguments on the relative rates of protein evolution on the X chromosome and on autosomes[100], many investigators have searched for empirical evidence for the prediction that X-linked genes should evolve faster than autosomal genes, a situation often referred to as the faster-X effect. This prediction originates from the idea that new beneficial mutations that are recessive will be directly selected for in the heterogametic sex when X-linked, increasing the rate of adaptive evolution. When autosomal, selection will only affect such mutations when they have drifted to

Haldane's rule
The tendency for offspring of the heterogametic sex to suffer more severe fitness consequences from an interspecific mating (hybridization) than the homogametic sex.

Effective population size
(N_e). Indicates how many individuals actually contribute alleles to the next generation, as opposed to the actual number of individuals in a population. For various reasons, including the preferential reproduction of some individuals and population size fluctuations over time, the effective population size is typically smaller than the actual number of individuals in the population.

Muller's ratchet
A population of non-recombining chromosomes may — by chance or by selection for beneficial variants contained within other haplotypes — irreversibly lose the class of chromosomes that is least loaded with deleterious mutations. Like a ratchet, this is a unidirectional process that inevitably leads to the degeneration of non-recombining chromosomes.

a sufficient frequency to start appearing in homozygotic form. For dominant mutations that are weakly deleterious, the smaller N_e of the X chromosome compared with that of autosomes means that the fixation rate of such mutations is expected to be higher on the X chromosome than on autosomes. This too could contribute to a faster X effect.

There is mixed support for a faster X effect from studies that have quantified protein evolution in different regions in the genome. The strongest evidence for its existence is from mammals, whereas in *Drosophila* the interpretation has either been an absense of or a weak faster X effect (for a review see REF. 101). In birds, there is clear support for an effect — in this case for a faster Z[102]. It has been concluded that genetic drift of slightly deleterious mutation is the main source of the faster Z effect[103], and this may be particularly relevant in systems of female heterogamety in which the difference in N_e between the Z chromosome and autosomes is exaggerated by polygynous mating. There are several possible confounding factors that may conceal or reinforce an expected signal of a faster X effect and the relative importance of these may depend on the characteristics of species. Two factors that should be considered are an organism's overall N_e and the gene content of the X (or Z) chromosome[101]. Because selection will be more efficient in larger populations, the proportion of slightly deleterious mutations that contributes to the accumulation of non-synonymous substitution on the X (or Z) chromosome is expected to decrease with increasing overall N_e, counteracting a faster X effect. When it comes to gene content, the non-random and varying distribution of rapidly evolving, sex-linked reproductive genes in XY and ZW systems should also be expected to affect the faster X or Z phenomenon.

With three X chromosomes for every four homologous autosomes per breeding pair, the N_e of X linked sequences should be three-quarters of that of autosomes. As a result, diversity levels are expected to scale accordingly. In practice, the N_e of males and females may differ owing to sexual selection and this will affect the neutral predictions for relative diversity levels on the X chromosome and the autosomes. This leads to an interesting contrast between the expectations for male and female heterogametic systems[104]: under male heterogamety the ratio of N_e for X-linked and autosomal sequences will approach 1 as the ratio of the number of reproducing males and females decreases. As an example, when there is an extreme variance in male reproductive success such that one male monopolizes ten females, there will be 21 X chromosomes and 22 autosomes transmitted by such mating (X/A ratio of 0.95). Under female heterogamety, the corresponding scenario means that there are 12 Z chromosomes and 22 autosomes transmitted (Z/A ratio of 0.55), and the ratio will approach 0.5 as the ratio of reproducing males and females decreases. These predictions remain to be empirically tested by the analysis of multiple species that vary in mating behaviour; if they can be confirmed they would provide an interesting link between mating system and genomic diversity, which would, in turn, have implications for the potential for evolution from standing genetic variation. Sex-specific patterns of migration will further affect the relative N_e of sex chromosomes and autosomes[105], as will demography — for example, population bottlenecks[106]. Moreover, it should be emphasized that this concerns neutral predictions and any difference in selection regimes on sex chromosomes and autosomes will clearly affect the overall predictions of genetic diversity[107].

Conclusions

The two most important conclusions that can be made from recent genetic studies of sex chromosomes are that these chromosomes are labile entities that change rapidly during evolution, and that the organization and content of sex chromosomes and the molecular evolution of sex-linked genes differs from that of autosomes and autosomal genes. Frequent switches between male and female heterogamety, the evolution of new sex chromosomes and distinct differences in chromosomal structure between even closely related species testify to the lability of sex chromosomes. Recent work has also shown that sex chromosomes have an unusual content of genes with sex-biased and/or tissue-specific expression and that extensive arrays of multi-copy genes with testis-specific expression occur on both sex chromosomes in at least some well-characterized systems.

So what questions remain to be addressed? For one thing, we do not know why some organisms evolve male heterogamety and others female heterogamety. A meta-analysis of the occurrence of the respective types in relation to, for example, life-history characteristics would be welcomed. Moreover, we still lack the complete sequence of a W chromosome in a system of female heterogamety and obtaining this will be important for understanding the evolution of non-recombining sex chromosomes that are not subject to the potent forces of sexual selection. Will we find similar ampliconic and palindromic structures to those on mammalian Y chromosomes, although in this case filled with ovary-specific genes? In terms of the evolutionary processes that lead to the differentiation of proto-sex chromosomes and the subsequent degeneration of non-recombining chromosomes, focus may switch to systems of incipient or more recently evolved sex chromosomes. In such systems, we may have the chance to study ongoing processes and use genetic data more directly to infer which forces drive sex-chromosome evolution. On a related note, population-genomic approaches based on deep sequencing of multiple individuals will make analyses of allele-frequency distributions possible, yielding information that is necessary to understanding the role that natural selection has in shaping diversity levels. Finally, although new or recently developed next-generation sequencing technologies will clearly be important for data acquisition on scales that were unrealistic just a few years ago, physical mapping will remain a key component to elucidating sex-chromosome organization and structure, in particular when it comes to repetitive structures. Specifically, cytogenetic techniques based on fluorescence *in situ* hybridization, including chromosome painting, will be necessary to reveal the homologies and origins of sex chromosomes.

Hill–Robertson interference
The general concept of selection at one locus affecting the efficiency of selection at a linked locus.

Selective sweep
A positive selection for an advantageous allele will increase the frequency of not only that allele but also other alleles contained within the same haplotype, causing a selective sweep (also referred to as genetic hitch-hiking). A hallmark of such sweeps is reduced levels of genetic diversity around the selected locus.

Background selection
Purifying (negative) selection against a deleterious allele will also tend to remove linked variants, or at least decrease their frequency, contributing to loss of genetic diversity (compare with selective sweep).

Chromosome painting
The use of an isolated chromosome, labelled with a fluorophore, as a probe in hybridization to a chromosome spread of the same or of a different species. The chromosomal regions homologous to the probe will be 'painted' and light up when fluorescence is detected.

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Competing interests statement
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