Leading Edge
Review
Cell

Sex Chromosome Specialization and Degeneration in Mammals

Jennifer A. Marshall Graves[1,*]
[1]Research School of Biological Sciences, The Australian National University, Canberra, ACT 2601, Australia
*Contact: jenny.graves@anu.edu.au
DOI 10.1016/j.cell.2006.02.024

Sex chromosomes—particularly the human Y—have been a source of fascination for decades because of their unique transmission patterns and their peculiar cytology. The outpouring of genomic data confirms that their atypical structure and gene composition break the rules of genome organization, function, and evolution. The X has been shaped by dosage differences to have a biased gene content and to be subject to inactivation in females. The Y chromosome seems to be a product of a perverse evolutionary process that does not select the fittest Y, which may cause its degradation and ultimate extinction.

Introduction

Chromosomal sex determination is widespread, but not ubiquitous, in the animal kingdom. In most vertebrate species with genetically determined sex, no differentiated sex chromosomes can be distinguished, although genetic differences may sometimes be identified. Animals with cytologically differentiated sex chromosomes may show male heterogamety (XX female:XY male) or female heterogamety (ZW female and ZZ male).

XY systems in which the X is large and gene rich and the Y small and heterochromatic are represented in species as diverse as humans, skinks, *Drosophila*, and even some plants. ZW systems include birds and snakes as well as butterflies. The mammalian XY and the bird ZW are superficially similar, with one partner (X or Z) large and gene rich, and the other (Y or W) small, heterochromatic, and almost devoid of genes. However, the gene content of these XY and ZW pairs are completely different (Nanda et al., 1999), and their superficial similarity is the result of a genetic imperative to degrade the heterogametic element.

The human Y chromosome has excited particular attention because of its small size, and the paucity and specialization of genes it bears. The theory that the Y chromosome degrades rapidly—and can even be completely lost—is supported by comparative studies in insects and vertebrates including mammals. A crude calculation of the average rate of loss of active genes from the human Y (Aitken and Graves, 2002) predicted its extinction in 10 million years or so, a gloomy outlook that has been vigorously debated.

H.J. Muller (Muller, 1914) first suggested that sex chromosome pairs evolved from a pair of autosomes, and Ohno (1967) developed this idea to explain variations in the snake ZW chromosome pair. This model can just as well be applied to the differentiation of the mammalian XY (Figure 1). One member of the pair acquired a sex-determining gene and became the sex-specific partner. Other male-advantage alleles accumulated, and recombination was suppressed. Mutation and deletion in the nonrecombining region rapidly degraded the sex-specific chromosome. This theory explains why the human Y chromosome shares a "pseudoautosomal" region with the much larger X, and why only 45 unique coding genes, largely related to genes on the X, remain on the human Y. It also explains why the Y has specialized genes that are testis specific and function in sex determination and spermatogenesis.

Why does the Y degrade? Is the human Y really disappearing, or is it simply the target of propaganda aimed at belittling men? Would the disappearance of the Y mean the disappearance of males? Here I shall explore the origin and organization of the human sex chromosomes and the degradation of the Y—focusing on factors that might speed up or slow down degradation and loss of the Y—and on the possible outcomes of loss of the Y.

Mammalian X and Y Chromosomes

Mammalian sex chromosomes are highly dimorphic (Figure 1). The large gene rich X and the small heterochromatic Y are almost completely differentiated but pair over a small homologous region at one tip (the pseudoautosomal region).

The human X (165 Mb) bears about 1000 genes with a variety of general and specialized functions (Ross et al., 2005). The X is highly conserved between species of placental mammal: complete sequencing of the X from mouse and human confirms that the placental X bears a virtually identical set of genes.

The human Y chromosome is much smaller (~60 Mb) and contains few genes. Complete sequencing of the euchromatic region revealed a total of 178 transcribed units (Skaletsky et al., 2003), but many are pseudogenes or amplified copies. The Y encodes only 45 unique pro-

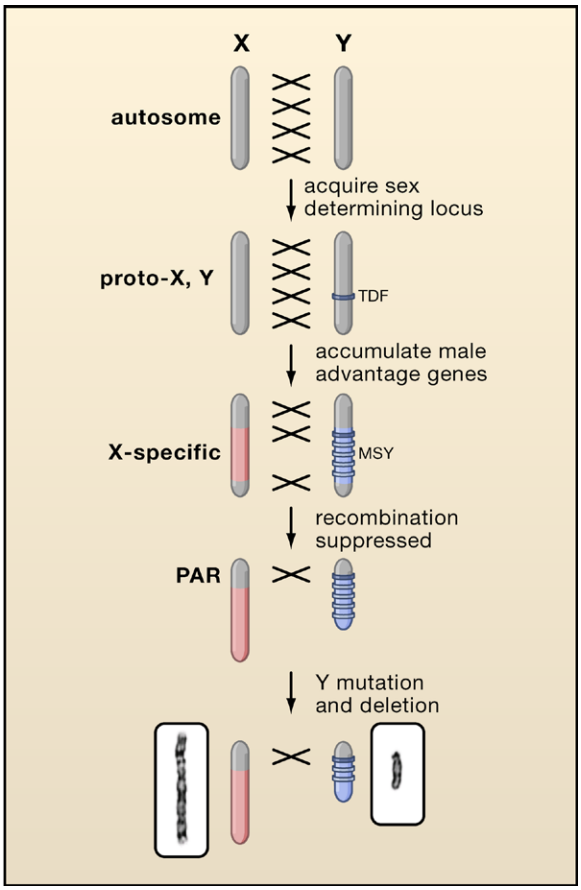

Figure 1. Differentiation of an X and Y Chromosome from an Ancient Autosome

This process is initiated when one partner acquires a sex-determining locus such as the testis-determining factor (TDF). Accumulation of male-specific alleles selects for repression of recombination (represented by crosses), creating an X-specific region and a male-specific region on the Y (MSY). Exclusion from recombination leads to rapid degradation of the MSY leaving only a small pseudoautosomal region (PAR). Active genes are lost, leaving largely genes that have, or acquire, a male advantage. This model accounts for the differences in size and gene content of the human X (left) and Y (right).

teins. The Y is also unusual in the "functional coherence" of the genes it bears, many of which have functions in sex or fertility (Lahn and Page, 1997). The human Y is replete with repetitive sequences of diverse origins, and many multicopy gene arrays are embedded in palindromes (Skaletsky et al., 2003; Rozen et al., 2003). Half of the heterochromatic long arm is composed of simple repeats with no coding function and apparently no phenotypic effect if deleted.

The mouse Y presents an even greater dichotomy, with all the well-characterized genes huddled together in a tiny short arm (http://www.ncbi.nlm.nih.gov/mapview/maps.cgi?taxid=10090&CHR=Y) and a long arm full of repetitive sequence. However, deletions of the long arm affect fertility, and Burgoyne and his collaborators have recently described active testis-specific genes buried in enormous repeats on the long arm of the mouse Y chromosome (Touré et al., 2005).

Origin of the Human XY Pair

The origin of the human XY pair can be traced back by comparison with distantly related mammals and other vertebrate classes. Birds, reptiles, and fish present a great variety of sex-determining systems. There are many fish and reptile species that use environmental cues like egg incubation temperature to determine whether the hatchlings develop as males or females. Other species have genetic sex determination but no distinguishable sex chromosomes, and relatively few have XY or ZW systems. None of these sex chromosome systems has homology to the mammalian XY pair.

What was the raw material for the mammalian XY system? Like all vertebrates, ancestral mammals undoubtedly had differentiated males and females. It has been suggested that the XY system may have arisen de novo in an ancestral mammal with temperature-dependent sex determination when an autosome acquired a sex-determining gene that overrode the sex-determining effect of temperature. Indeed, we can deduce that the genes on the human X chromosome are present as blocks on autosomes of a marine turtle with temperature-dependent sex determination (Graves and Shetty, 2001) (Figure 2A), whose karyotype is almost identical to that of a chicken and may resemble that of our common ancestor 300 million years ago (MYA).

However, this hypothesis is challenged by new findings in the platypus, a monotreme mammal with 5X and 5Y chromosomes. These ten sex chromosomes form a translocation chain at meiosis (Grützner et al., 2004). At one end is an X chromosome bearing genes typical of the mammalian XY chromosome pair. At the other end is an X containing the *DMRT1* gene that lies on the bird Z chromosome and is a candidate for bird sex determination (Figure 2B). It seems unlikely that this association is purely accidental and could mean that the *SRY*-driven mammalian XY system may have taken over from an ancestral reptile/bird ZW system. This may have occurred by a Z-autosome translocation in a ZZ (male) animal, and the acquisition of a male-dominant sex-determining gene on the autosome, though there are other possibilities permitted in this multiple sex chromosome system (reviewed by Grützner et al., 2006).

In a search for the building blocks of the XY pair, the gene content of the X has been compared between placental mammals and the other two major mammal groups, marsupials (which diverged from placental mammals 180 MYA) and monotremes (210 MYA). This showed very clearly that the X (and therefore the Y) comprises ancient and added regions (Graves, 1995) (Figure 3). An ancient region (equivalent to the long arm of the human X and the region just above the centromere) is shared by all mammals and is clearly distinguished by chromosome painting, a technique in which marsupial X chromosome DNA is isolated, tagged with a fluorescent

dye, and hybridized to the homologous sequences on the human X (Glas et al., 1999) (Figure 2C). However, a large region (equivalent to the rest of the short arm and including the pseudoautosomal region) is autosomal in marsupials and monotremes, implying that it was added to the placental X between 100 and 180 MYA. The human Y chromosome, too, is composed of a corresponding ancient region shared with marsupials, and an added region, on the Y only in placental mammals (Waters et al., 2001). Thus the human X is technically a neo-X, and the human Y is a neo-Y.

Birds do not share the mammal XY system, but the origins of the XY pair can be traced further back in time by mapping human X genes to large blocks on chicken autosomes (Nanda et al., 1999). The complete sequencing of the genomes of chicken and three fish species allowed a detailed comparison of gene arrangement between birds and mammals (Kohn et al., 2004) (Figure 3). Genes in the recently added region of the human X lie, in the same order, on chicken chromosome 1. Genes in the ancient conserved region of the human X lie largely on chicken chromosome 4p, which is a microchromosome in other birds and the short arm of chromosome 8 in marine turtles. However, two small parts of the conserved region of the human X (the region near the centromere; Xp and a gene-rich region near the end of the long arm) contain genes that map to chicken chromosome 12 and other microchromosomes and are on a separate chromosome also in fish. These three autosomal regions must represent separate ancestral building blocks that fused to form mammalian sex chromosomes. They were put together progressively; evolutionary layers 1 and 2 fused 310–210 MYA, and layer 3 was added 180–100 MYA in placental mammals (Figure 3). The Y, too, contains genes representing each of the three building blocks.

The same three layers are also distinguished by a completely independent metric—differences in the divergence time between genes shared on the X and Y (Lahn and Page, 1999) (Figure 3). Genes in evolutionary layer 1 show the most divergence between Y-borne genes and their X homologs, so they must have diverged first. This layer contains the sex-determining gene *SRY*, which became Y specific about 250 MYA, consistent with a key role in initiating differentiation of the X and Y. Layer 2 coincides with a group of genes with intermediate XY divergence, and layer 3 can be subdivided into two layers with lesser degrees of divergence. The pseudoautosomal region (with zero divergence) constitutes a fifth layer. Lahn's and Page's (1997) suggestion that these five "geological layers" were defined by internal rearrangement clearly does not apply to the three building blocks of the X, which were separate in a vertebrate ancestor. However, internal rearrangement may well have established the subdivisions within the recently added region.

Ancestry of the different regions of the mammal X and Y has implications for the gene content of the sex chromosome (described below) and even gene regulation. For instance, many genes on the human X were found to escape X inactivation, the mechanism that compensates for the dosage difference between XY males and XX females. Reasons why these genes needed to be present in duplicate in females were sought, but the finding that these escapees cluster on the recently added region of human

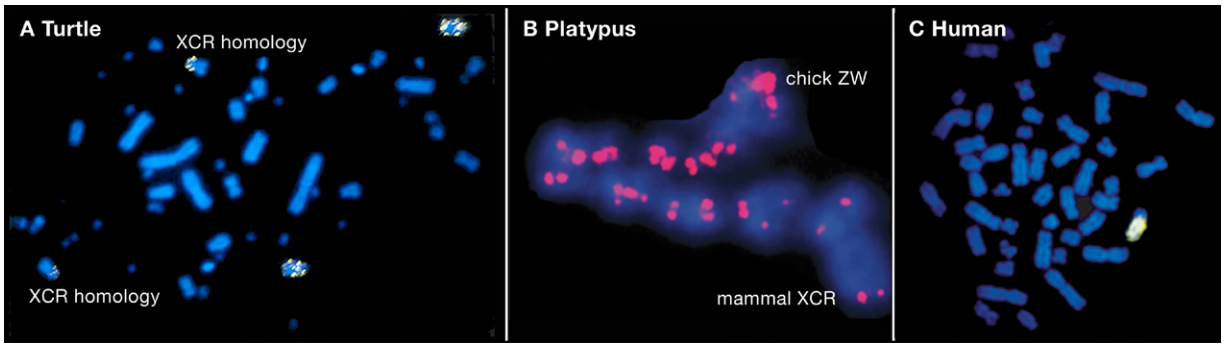

Figure 2. Origin of the Human Sex Chromosomes

(A) The chromosomes of a common ancestor of reptiles, birds, and mammals might have had a karyotype like this temperature sex determined marine turtle, which has no sex chromosomes. Fluorescence in situ hybridization (FISH) using DNA from chicken chromosome 4p (homologous to a region of the human X; Xq) reveals that this ancient conserved region of the X chromosome (XCR) is homologous to the short arm of turtle chromosome 8 (the partial chromosome signal). Chicken chromosome 4q (which is homologous to human autosomes) hybridizes to the entire turtle chromosome 5 (the whole chromosome signal). In other experiments (not shown here), the part of chicken chromosome 1q homologous to the human X added region hybridizes to the same part of turtle chromosome 1.

(B) The platypus has 5X and 5Y chromosomes that link up in a translocation chain at meiosis; the ends of each chromosome are marked by a probe (pink spots) that hybridizes with telomeres. The X at one end has homology to the conserved region of the mammal X and the X at the other end shares genes with the chicken ZW system, suggesting that the mammal XY system evolved in a reptile ancestor with an ancient ZW system (Grützner et al., 2004), rather than both systems evolving independently from an ancestor with temperature-dependent sex.

(C) FISH with a physically isolated kangaroo X hybridized to human metaphase chromosomes. It hybridizes to only the long arm and pericentric region of the human X, identifying the ancient region of the human X.

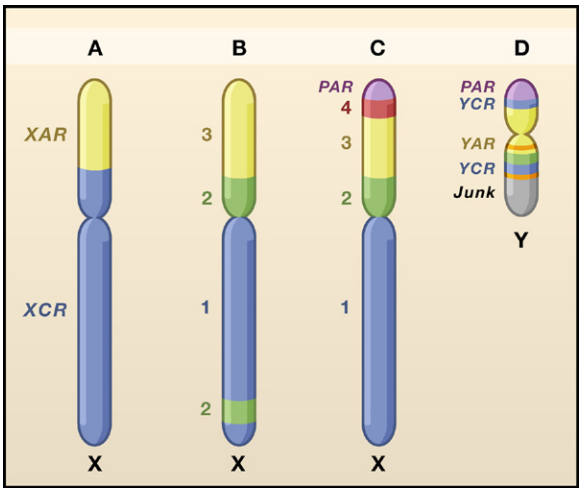

Figure 3. Evolutionary Layers of the Human X and Y

(A) Comparative mapping of the orthologs of genes from other mammals on the human X detected an ancient region conserved on the X in all mammals (blue, XCR) and an added region (yellow, XAR) that is autosomal in marsupials and monotremes so was added to the X 180–100 MYA.

(B) Chicken homologs of human X genes map to three autosomal regions by comparative mapping. This analysis identifies three evolutionary layers that correspond with XAR (layer 3) and subdivide XCR (layers 1 and 2).

(C) Comparisons of nucleotide sequence between genes on the human Y and their partners on the X groups genes into five clusters. The oldest cluster mostly corresponds to layer 1, and the next oldest to layer 2. Layer 3 (XAR) contains two clusters and the PAR.

(D) By direct comparative mapping of the human and marsupial Y, and by analogy to the evolutionary layers of the X, we can deduce that the human Y chromosome, too, is subdivided into corresponding ancient and added regions. The ancient conserved region (YCR, blue) is tiny, and most of the Y derives from the added region (YAR). A few genes (orange) have also been transposed from other chromosomes.

Xp (Carrel and Willard, 2005) suggests that this region has simply not had time to be fully recruited to the X inactivation system. This provides a particularly good example of how biological differences may have their explanation in genome evolution rather than biochemistry.

Evolution from an autosomal pair predicts that the mammalian X and Y started out with an unremarkable assortment of genes that are still going about their autosomal business in other vertebrates. How have the gene contents of the X and Y been shaped by their differential representation in males and females?

The Evolution of X Chromosome Inactivation

One consequence of the degeneration of the Y chromosome is the unequal dosage between males and females for X-borne genes with no Y partners. The necessity for dosage compensation of genes on the X has selected for a variety of dosage compensation mechanisms in invertebrates and vertebrates. In mammals, a system has evolved in which one of the two X chromosomes in a female becomes transcriptionally silent in the embryo and maintains this silence stably throughout life. How did this system evolve?

Regions of the X are thought to have been recruited into the X chromosome inactivation system as their Y partner became degraded (Graves and Schmidt, 1992; Graves et al., 1998; Jegalian and Page, 1998). This hypothesis is supported by the observation that many genes on the human X escape inactivation, although they have no active Y partner (Carrel and Willard, 2005). The clustering of these escapees in domains in the recently added layer of the X suggests that the process lags behind Y degradation and is not yet completed in the newest layer. In some patches of the human Xp, several genes escape X inactivation, suggesting that dosage compensation is not an urgent requirement of many genes (as is clear from the normal phenotype of heterozygotes for null mutations and even deletions). I suggest that loss of active genes from the Y may proceed for some time until one is reached that has an immediate deleterious dosage effect, when there is rapid selection for incorporation of the whole domain into the X chromosome inactivation system.

The molecular mechanism of this extraordinary epigenetic silencing system is complex, involving binding with an untranslated RNA, then accumulation of modified and variant histones, and DNA methylation (Heard, 2004). The stages by which such a complex was built up can be teased apart by examining X chromosome inactivation in distantly related mammals. Marsupials show X chromosome inactivation, but whereas placental inactivation is random and very stable, marsupial inactivation is paternal, incomplete, and tissue specific; the molecular mechanism appears to be less complex, lacking DNA methylation but sharing histone modification (Cooper et al., 1993; Wakefield et al., 1997), and may reflect an ancestral state. Molecular studies of this simpler system will be very informative.

The Biased Gene Content of Mammalian X Chromosomes

The theory that differentiation of the X and Y occurred by degradation of the Y implied that the content and function of genes on the X should reflect that of the ancestral autosome. This may be quite wrong; genome sequencing has confirmed the gathering suspicion that the gene content of the human X, as well as the Y, has been strongly shaped by special selective forces.

An early analysis of the function of genes mapped to the human X concluded that genes involved in sex and reproduction are greatly overrepresented compared to other genes (such as those involved in formation of the skeleton) (Saifi and Chandra, 1999), and this enrichment of sex and reproduction genes was supported by the finding that genes expressed in mouse spermatogonia are overrepresented on the mouse X (Wang et al., 2001). An accumulation of male-advantage genes on the X has been suggested to result from rapid selection in the hemizygous male, even if they are neutral or detrimental in females (Rice, 1987a). A new recessive allele that enhances gonad or sperm function will be expressed

in males and immediately selected, but it will have no effect on the phenotype of heterozygous females, and there will be few homozygotes to worry about as long as the allele is rare. When it becomes common, deleterious effects could be mitigated by restricting the expression of the gene to the testis. However, dominant alleles will be twice as frequent in females so it might be expected that female-advantage genes, too, accumulate on the X.

This theory predicts that male-advantage genes should accumulate on the X in all XX female:XY male animals, so consternation greeted the discovery that in the fruit fly *Drosophila melanogaster*, the X seems to be depleted in genes with male-biased expression, and the same is true of the worm *Caenorhabditis elegans*, which has an XX female:XO hermaphrodite sex chromosome system (reviewed in Reinke, 2004). It was suggested that this "demasculinization" of the X led to germline inactivation, driven by sexual antagonism (Wu and Xu, 2003). These apparently contradictory data were resolved by studies of gene expression during mouse meiosis, which showed that genes expressed in early (mitotic) stages of spermatogenesis are overrepresented on the mouse X, but genes expressed later are depleted and meiotic-specific genes are completely absent. This makes sense because the mammalian X chromosome becomes inactive early in male meiosis, making the X a bad place for genes with critical functions in late meiosis. Indeed, many mammalian X-borne genes coding for housekeeping enzymes that are vital in spermatogenesis (such as *PGK1*) have spawned autosomal paralogs like the testis-specific *PGK2* (McCarrey and Thomas, 1987). Thus the distribution of genes on the mouse X is a compromise between a strong drive to accumulate male-advantage fertility genes and their depletion due to X inactivation. In *Drosophila* and *C. elegans*, sex chromosome inactivation occurs much earlier in meiosis, so any tendency to accumulate male-specific genes was opposed by strong selection for their loss from the X.

In addition, the human X may be responsible for a disproportionate number of mental retardation syndromes caused by mutations in genes on the X that are required for brain development or function (Zechner et al., 2001). Genome sequencing confirms that these "intelligence" genes are in 5-fold excess on the X. The reason why the X might have accumulated genes involved in brain development and function is more speculative—is intelligence a male-advantage trait? Or is it sexually selected by females, perhaps (like the peacock's tail) as a marker for good genes (reviewed in Graves et al., 2002)? Sexual selection is extremely rapid and could explain the rapid increase in brain size in hominids.

What was the origin of the sex and reproduction and intelligence genes that accumulated on the X? There are three possibilities. One is that these genes were recruited to the X from other locations in the genome. This seems unlikely, given that the gene content of the building blocks seems to have changed little in the 310 MY since the divergence of chicken and humans. More likely is that genes on the X with a general function in both sexes acquired new roles as a result of sex-specific selection. Alternatively, an autosome particularly well-endowed with these genes might have been "chosen" for a career as a sex chromosome. These ideas could be tested by examining the function of the autosomal homologs of mammalian X-borne genes in chickens and kangaroos.

A venerable and puzzling observation is that many genes on the X contribute to both reproduction and intelligence, as seen by the many X-linked mental retardation syndromes that are accompanied by gonadal abnormalities. This coincidence of functions is hard to explain by any developmental similarity in testis and brain, and the "brains and balls" coincidence has been subject to ongoing debate. However, it could be easily explained just by the propensity for genes with wide expression and general function (for example, in chromatin remodeling like *SOX3* and *ATRX*, or RNA metabolism like *RBMX*) to serve as raw material for two quite different selective forces that shape the same protein into a factor vital for both brain and gonad development.

The "smart and sexy" mammalian X has been dubbed "the engine of speciation" (Graves et al., 2002) because its propensity to accumulate genes that affect reproduction and intelligence could provide pre-mating and post-mating reproductive barriers that are critical to mammal speciation process. The *Drosophila* X is known to have an important effect on hybrid inviability and is disproportionately involved in speciation (Orr and Coyne, 1989).

Specialization of the Y

The gene content of the human Y is even more biased than that of the X, and its "functional coherence" (Lahn and Page, 1997) is completely unique in the genome. I will briefly describe genes on the Y of humans and other mammals, before discussing their peculiarity.

The most important function of the mammalian Y is sex determination, and the first genes identified on the Y included candidates for the testis-determining gene, *SRY* (Sinclair et al., 1990), and a zinc finger gene *ZFY* that was the original candidate for this role (Page et al., 1987). Other genes were identified that were testis specific and suspected to have functions in spermatogenesis, but several were found that were ubiquitously expressed and had no obvious male-specific function.

The differences in properties of genes on the Y lead to the suggestion of two discrete classes of Y genes with different functions and evolutionary history (Lahn and Page, 1997). Class I genes like *ZFY* were single copy, ubiquitously expressed genes that were simply relics of ancient homology with the X. Class II genes were multicopy and testis specific with no X partners. These interesting genes were suggested to have been acquired from autosomes because they conferred a selective advantage to males, much as fertility factors appear to have accumulated on the *Drosophila* Y (Carvalho, 2002).

However, a class I/II dichotomy does not stand up to

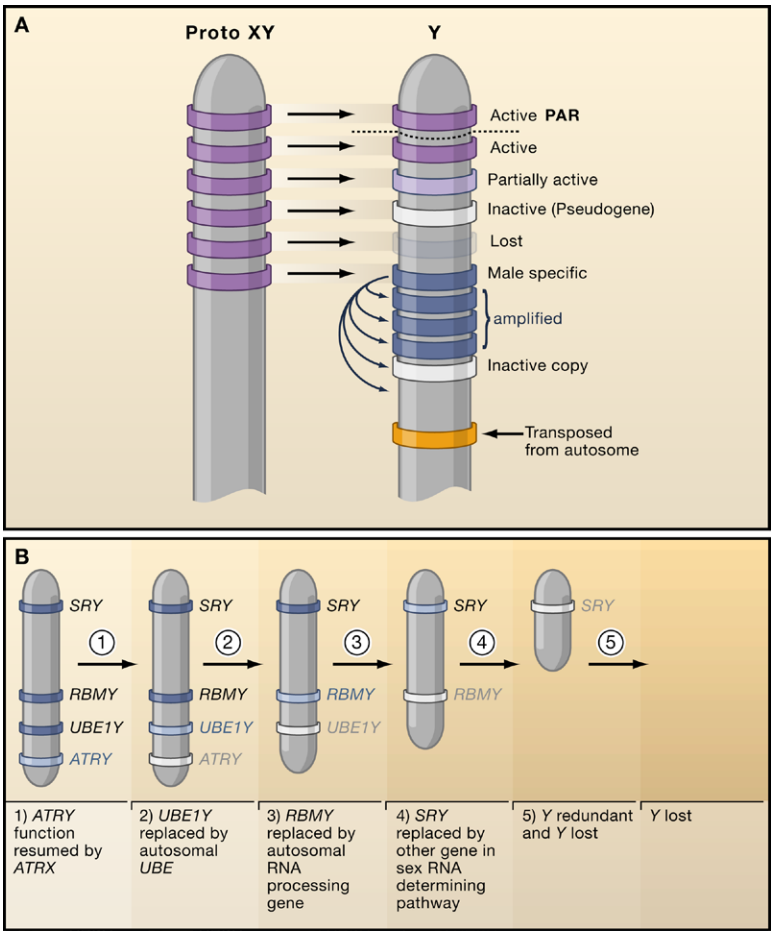

Figure 4. Fate of Genes on the Y

(A) An evolutionary continuum—some MSY genes remain active (purple) and some partially active (light purple), but most are inactivated (white) and deleted. A few genes acquire a male-specific function in sex or spermatogenesis (dark blue); many of these are amplified (indicated by arrows), then amplified copies may be degraded. Some genes are transposed from autosomes (orange).

(B) Terminal stage of Y degradation. Even genes under positive selection may be lost as their function is taken over by other genes. Ultimately all the critical male-specific genes could be lost from the Y, leaving it redundant and liable to complete loss, as in several rodent species.

examination. Several genes with testis-specific expression and functions in fertility were also discovered to have X partners from which they obviously evolved. For instance, the multicopy testis-specific *RBMY* gene that is required for spermatogenesis in humans clearly evolved from a ubiquitously expressed gene *RBMX* (Delbridge et al., 1999) that appears to have a function in brain development (Tsend-Ayush et al., 2005), and the multicopy testis-specific *TSPY* gene is homologous to the widely expressed *TSPX* that has homology to a cell-cycle gene (Delbridge et al., 2004). The testis-specific marsupial *ATRY* gene evolved from a widely expressed X-borne gene *ATRX*, mutations in which cause α thalassemia, mental retardation, and sex reversal in humans (Pask et al., 2000). Even *SRY* was found to have a widely expressed X homolog *SOX3* (Foster and Graves, 1994), which seems to be involved with X-linked mental retardation and pituitary function (Laumonnier et al., 2002).

It appears, therefore, that there are *not* two discrete classes of Y-borne genes, nor even three as was subsequently suggested (Lahn et al., 2001); rather most of the genes on the Y form an evolutionary continuum, representing all degrees of degradation and divergence from the X-borne predecessor (Figure 4A). Most genes became inactive and were rapidly lost from the Y, and a few pseudogenes are still clinging on in various stages of degradation. A few Y genes had or acquired a sex-specific function that ensured their survival. Many of these have been amplified, presumably to preserve sufficient function despite the forces that mutate and inactivate them.

What changes honed ubiquitously expressed genes with functions in both sexes into a testis-expressed gene with a function in fertility? Sequence comparisons between the coding regions of *RBMX* and *RBMY* show many changes including loss and amplification of exons (Delbridge et al., 1999). The ubiquitously expressed *ATRX* gene (mentioned above) has a testis-expressed homolog on the marsupial (but not the placental) Y; this change in expression and presumably function was accompanied by changes in many domains that would alter the binding of this complex protein to several factors that affect chromatin condensation (Park et al., 2005). *SRY* appears to be a truncated version of its ancestor *SOX3* and was left with little besides the region that binds and bends DNA. This suggests that *SRY* might act indirectly on testis determination, perhaps by interfering with the binding of another chromatin remodeling protein.

The few exceptional genes on the human Y with no obvious X homolog appear to have been transposed to the Y from autosomes. Two examples on the human Y may already have had a male-specific function in their original site. *DAZ*, a multicopy testis-specific spermatogenesis gene, is most closely related to an autosomal gene with an ancient function in reproduction (Saxena et al., 1996). The gene structure of the Y copy suggests that it was transposed from its autosomal site, and its absence from nonprimates suggests a recent arrival on the human Y. *CDY* appears to be a testis-specific retrotransposed copy of a ubiquitously expressed autosomal gene with no apparent function in spermatogenesis in

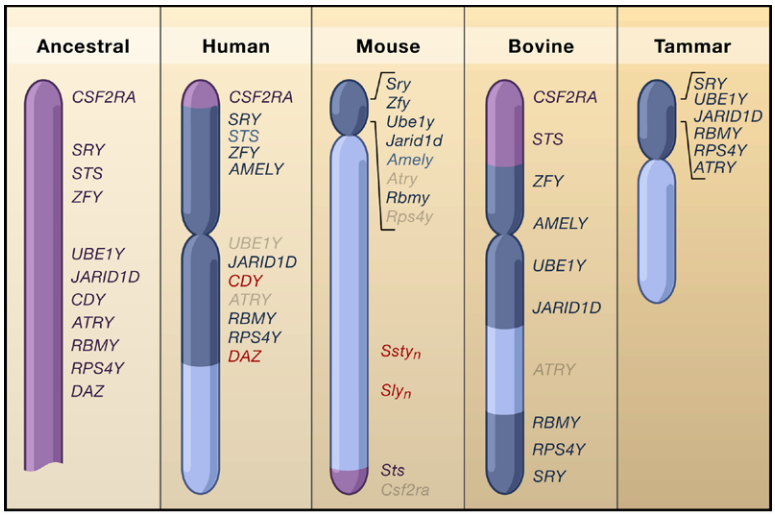

Figure 5. Genes on the Y in Different Mammals

Recent independent degradation of the Y from the ancestral proto-Y has left different subsets of active genes within the PAR and the MSY in different mammalian lineages. Shown are PAR genes (purple), active genes in the MSY (dark blue), pseudogenes (light blue), deleted genes (gray text), genes transposed from an autosome (orange). Even genes with a critical function in one species (such as *UBE1Y*) have been lost or inactivated in others.

humans or other mammals, although there is a testis-specific alternative transcript in mouse. It has been on the Y much longer, as it is shared by other mammals, which appear not to process the alternative testis-specific *CDY* transcript, perhaps because its function has been taken over by the Y-specific gene (Dorus et al., 2003). Therefore, although it is evidently not common, the Y can certainly appropriate male-advantage genes from autosomes in mammals (Hurst, 1994), just as in *Drosophila* (Carvalho, 2002).

Thus most of the genes on the human Y appear to be relics of genes on the X, favoring a "wimpy Y" model. They present examples of a continuum of degradation. Some appear to serve no male-specific function and are probably in the throes of degradation. Some have been retained because they acquired male-specific functions. At least a few male-advantage genes were acquired by a "selfish Y" from autosomes. Both processes have contributed to the evolution of a (selfish, wimpy?) human Y.

Evidence for Degeneration of the Mammalian Y

In mammals, accepting the theory that the Y represents a broken-down X means accepting that it has lost all but 45 of the ~1000 genes with which it began. In chicken, comparisons of the W and Z tell the same story: of all the genes on the chicken Z, only a few remain on the W (Fridolfsson et al., 1998).

Stages in degeneration are not obvious at the cytological level in the small and gene-poor mammalian Y but are obvious in the female-specific W chromosomes of birds and snakes. In fact, Ohno's realization (Ohno, 1967) that the different sizes of W chromosomes in different snake families represented stages in the degeneration of the heterogametic W was the inspiration for his theory of progressive degeneration of sex chromosomes. Although the Z chromosome is similar in all snake families, the W ranges from being virtually identical to the Z in pythons to extremely differentiated from the Z in vipers. Birds exhibit the two extremes of this spectrum, sharing a homologous Z chromosome but having a W that is largely homologous to the Z over its length in the ratites (flightless birds such as emus and ostriches) but is greatly reduced in size and gene content in carinate birds (including chicken) (Shetty et al., 1999).

However, stages in degeneration of the mammalian Y are obvious at the gene and sequence level, in the activity and function of the few genes that remain on the human Y. Examples may be found of every stage in a continuum of degradation (Figure 4A), from pseudoautosomal genes, fully active genes in the nonrecombining region (the male-specific region on the Y; MSY) with homology to their X partner (such as *ZFY*), partially active genes (for example, the tooth enamel gene amelogenin), pseudogenes (*STS*)—and a very long list of genes that have been completely deleted. In addition, there are examples of genes that have been re-tooled for a male-specific function (such as *SRY*), and of male-specific genes that have been amplified and several copies inactivated in turn (*RBMY*). There are also examples of genes with a function in spermatogenesis that have been copied onto the Y (such as *DAZ*).

Convincing evidence also comes from comparisons of the gene content of the Y in different mammal species. Y chromosomes of different species have lost different subsets of the same gene set represented on the X (Figure 5), as would be expected if they have been degenerating independently for the last 100 or 180 MY. For instance, *UBE1Y* is present in all marsupial and placental species except primates, in which only bits and pieces remain, whereas *RPS4Y* is present in marsupials and all placental mammals except rodents and *ATRY* has been retained only in marsupials (reviewed in Graves, 2002).

As well as the physical loss of genes, other processes that change the structure and function of the Y are tracked by comparing different mammal species. Although placental mammals have similar numbers of active genes on the Y, there are differences in the number that remain pseudoautosomal, and in their degree of specialization. The pseudoautosomal region has been reduced in primates and further reduced in rodents

(Figure 5). For instance, the *STS* gene is in the pseudoautosomal region in carnivores and ungulates but is in the male-specific region of the Y in primates and is inactive in humans. Specialization appears to be more advanced in mouse, in which most genes on the Y are testis specific and their partners are subject to X inactivation. For instance, *ZFY* is ubiquitously expressed in humans but testis specific in mouse (reviewed in Graves, 2002).

The process by which the mammalian Y has degenerated has been much debated. Did degradation sweep across large regions of Y that lost affinity with the X because of some genomic cataclysm like an inversion? Or was it a creeping process by which gradual accumulation of sequence differences progressively and processively reduced recombination? Comparison of X and Y chromosomes from a variety of mammals provides evidence for both processes. Certainly there is at least one 30 million-year-old inversion on the human Y that is visible as a separate evolutionary layer on the X. However, there is also evidence of a more gradual process occurring within the blocks, which is visible as molecular tide marks left by waves of the receding pseudoautosomal region. Traces of ancient pseudoautosomal region boundaries are recognizable by sudden changes in XY sequence homology and GC content, even within genes. For instance, the gene that encodes amelogenin (tooth enamel) has evidently moved gradually from the pseudoautosomal region to the nonrecombining region, leaving signature changes in GC composition (Marais and Galtier, 2003).

More evidence of degradation comes from detailed before-and-after studies of species—vertebrate and invertebrate—in which autosomal regions were suddenly transformed into sex chromosomes.

Degradation of the Y—The First Stages

One of the biggest mysteries in biology is what drives degeneration of nonrecombining regions of the Y, and why positive selection for male-advantage genes does not stop it.

All vertebrates have a male:female differentiation, so the evolution of a new sex-determining system amounts to a replacement of a preexisting system—either genetic or temperature. The first steps of sex chromosome differentiation are taken when a new sex-determining gene is acquired by an autosome, which instantaneously becomes a male-limited proto-Y. In mammals, the acquisition of the testis-determining factor *SRY* was not a physical gain but an allelic variant of the ancient gene *SOX3* (present in layer 1 of the X and with orthologs in birds and fish). In birds, an ancient dosage-sensitive gene *DMRT1* in a sex-determining pathway had evidently been pressed into service, and the autosome that bore it (represented by chromosome 4 in turtles; Graves and Shetty, 2001) became the bird Z.

Other alleles that confer a male advantage then accumulate near the new sex locus, a process that has been experimentally demonstrated (Rice, 1987b). Suppression of recombination with the proto-X in the region keeps the male sex-specific package together. In the nonrecombining region, mutation, deletion, and invasion of retrotransposons rapidly degrades the Y, leaving it devoid of active genes except for the few that have managed to acquire a vital sex-specific function.

To understand the first steps of degeneration, it would be a great advantage to study Y chromosomes at an early stage of differentiation. Unfortunately, the trail has gone a bit cold for our most familiar sex chromosome systems in which well-differentiated XY or ZW chromosomes have had hundreds of millions of years to diverge. However, there are valuable examples from other vertebrates and invertebrates in which sex chromosomes have been recently molded from autosomes, either by plopping a sex-determining locus onto an autosome or fusing a whole autosomal region onto a sex chromosome.

New sex chromosomes can be generated when an old sex-determining gene moves from chromosome to chromosome. A spectacular example is the fly *Megaselia* (Traut and Willhoeft, 1990), in which the sex-determining gene is part of a transposable element that moved to different chromosomes in different populations. Movement is so frequent that little degeneration occurs near the sites of insertion.

Two lovely examples in which acquisition of a sex-determining gene created a nascent Y chromosome have been described in fish species that have genetic sex determination but no cytologically distinguishable sex chromosomes. A sex-linked enzyme in the three-spined stickleback provided a pointer to a terminal chromosome region that harbors the male-determining locus. Recombination was shown to be suppressed over the flanking 250 kb region, which showed extensive divergence between the nascent X and Y due to insertions and deletions in the proto-Y (Peichel et al., 2004). These genes were not sex-linked in closely related species, suggesting recent evolution of a new sex region. A new male-determining gene in medaka has been identified as a homolog of *DMRT1*, a dose-dependent gene involved in sex in a variety of vertebrates and invertebrates. An extra *DMRT1* copy was evidently transposed onto an autosome (Kondo et al., 2004), creating a proto-Y that diverged over a 260 kb male-specific region. The absence of this male-specific region from closely related species dates the arrival of the new gene only 4 MYA.

There are many reptile groups that contain genetic and temperature sex-determining species. A hunt for nascent sex chromosomes was expected to identify new genetic sex-determining systems that evolved on a temperature sex-determining background, as suggested for the mammalian XY system. However, a genetic sex-determining dragon lizard species with temperature sex-determining relatives proved to have a completely differentiated W microchromosome revealed by comparative genome hybridization, suggesting, instead,

a recent switch to temperature sex determining by an ancient genetic sex-determining system (Ezaz et al., 2005). Switches between genetic sex determining and temperature sex determining therefore probably go in both directions. Indeed, some species combine both mechanisms under different environmental conditions, and it is now becoming clear that genetic sex determining and temperature sex determining are the ends of a continuum (Sarre et al., 2004).

The opposite process, whereby an autosomal region has been dragged into the sex pair, has occurred many times to create a neo-X or neo-Y or both. There are many such in mammals, including African pygmy mice (Veyrunes et al., 2004), many marsupials (Hayman and Sharp, 1981), as well as humans and other placentals, but these are too old to provide information about the first events of Y differentiation. A variety of neo-Y chromosomes of various ages can be studied in *Drosophila* species (reviewed Charlesworth et al., 2005), but it turns out that change has happened so rapidly that even the most recent fusion has already passed well beyond the first steps and is relegated to the following section.

The Process of Y Degeneration

Comparisons between sex chromosomes in many taxa show that once a new sex-determining region has been established on a proto-Y, the nonrecombining region may enlarge rapidly and apparently inexorably as sequence on the Y is mutated, deleted, and invaded by repetitive elements. Many attempts have been made to explain what drives this degeneration, and why it is not more effectively countered by positive selection for male-advantage genes.

The neo-Y in some recently diverged *Drosophila* species provides the most detailed study of galloping degeneration (Charlesworth et al., 2005). For instance, fusion of the Y with an autosome occurred only 1 MY ago in *D. miranda*. This neo-Y, half original Y and half original autosome, has homologs in the Y and autosome of closely related species, so we can track the changes that occur in autosome material that was suddenly confined to the male lineage. Even after such a short time, a considerable region of the neo-Y is unable to recombine with its erstwhile partner, and sequence divergence is obvious. Genes were found to be mutated, inactivated and deleted, and junk inserted in more than half of the autosomal addition. Dosage compensation galloped along its erstwhile partner, the neo-X, to keep pace with the degradative changes. In other *Drosophila* species, the opposite occurred: the fusion of the X with an autosome. One copy of the original autosome, now confined to the male lineage, also shows signs of rapid degeneration along with the original Y.

Degeneration of the mammalian Y, even over hundreds of millions of years of evolution, could also be instructive at the molecular level now that the genomes of several mammals have been completely sequenced. However, the Y chromosome cannot be sequenced by the standard "shotgun" method because of its high repeat content. The human and chimpanzee Y chromosomes have been assembled by heroic BAC-based sequencing (Skaletsky et al., 2003; Hughes et al., 2005), and the sequence of 5.3 Mb of the X-Y shared male-specific region has been compared in detail. This region of the human Y contains 16 active genes and 11 pseudogenes, which are all represented in the corresponding region of the chimpanzee Y. However, only 11 of the 16 are active in chimpanzees, implying that a third of them became defunct within the last 6 MY of chimpanzee evolution. The opposite does not seem to be the case (although it is always difficult to assert the lack of unknown genes) since the 11 pseudogenes in this region of the human Y are inactive also in chimpanzee, and this region of the Y contains no genes that have homologs on the human X, from which the Y evolved. This suggests that the chimpanzee Y is degrading faster than the human Y.

Thus it seems that Y chromosomes in different taxa are all subject to degradation, but that the rate of degradation can vary widely. Why?

Forces that Degrade the Y Chromosome

What causes Y degradation? Many forces are lined up against the heterogametic sex chromosome. In mammals, these fall into two categories: a higher mutation rate, and the inefficiency of selection on a nonrecombining chromosome.

In mammals, the Y seems to be subject to far more mutation, deletion, and insertion than the rest of the genome. This bias, calculated as a factor of 4.8 in humans (Lindblad-Toh et al., 2005), accounts for the observation that most de novo dominant genetic diseases arise on the father's chromosomes (Makova and Li, 2002). This bias arises almost entirely because the Y must spend every generation in the hostile environment of the testis, whereas autosomes and the X cycle through the testis only half or a third as often. The testis is a dangerous place for a chromosome to be for two reasons. Firstly it takes many more cell divisions to make a sperm than an egg, providing additional opportunities for damage. Secondly, the sperm is an oxidative environment and lacks repair enzymes (Aitken and Graves, 2002). This heightened mutation rate is not shared by the female-specific W chromosome in chickens, in which the W always occupies the relaxed environment of the ovary (Ellegren and Fridolfsson, 1997).

In addition, the repetitive structure of the human Y chromosome makes deletions very frequent. For instance, recombination between homologous sequences in palindromes on the human Y frequently removes 6 or 7 Mb and several fertility genes. Remarkably, deleted Y chromosomes may survive and prosper: one family of Y chromosomes with an 1.8 Mb deletion that removes at least eight testis-specific gene families is widespread in Europe (Repping et al. 2004). Sykes (2003) suggests that such deletions become more and more frequent as sequences on the Y amplify and decay and predicts that deletion of the long arm of the human Y is accelerating.

Conversely, it has been suggested that the special structure of the human Y chromosome acts to retard degradation by permitting gene conversion between pairs of multicopy genes arrayed on opposite arms of the huge palindromic structures (Rozen et al., 2003). Could conversion of a mutant copy to a wild-type copy make up for the lack of recombination with the X? It seems to me that the answer must be "No" because conversion of a wild-type to a mutant copy must be equally as frequent as restoration of wild-type. That this process often resolves the wrong way is painfully evident from the arrays of NORFs—homogenized sequences that are transcribed in the testis but can no longer be translated—in palindromes. These appear to be dead gene clusters that have converted to mutant form. Thus conversion within palindromes can only increase the level of variation, and once more we must rely on selection to choose the fittest Y.

Why doesn't positive selection work better to protect the Y chromosome? Debate of this central question has raged for decades, and there are many theories (expertly reviewed by Charlesworth and Charlesworth, 2000). These theories all relate to the inability of the Y to recombine with the X and apply also to other nonrecombining sequences such as the mitochondrial genome. Absence of recombination means that the entire male-specific region of the Y is inherited as a unit, for good or ill, and it is subject, as a whole, to the vagaries of drift and selection. This dependence of genes on their neighbors (Hill-Robertson interference) means that selection does not work very efficiently. An advantageous new allele can be dragged into oblivion by its mediocre neighbors ("background selection"), or a deleterious allele can tag along with a successful new variant ("hitchhiking"). Hitchhiking could explain the selective sweeps that seem to have reduced variability on the human Y and driven particular Y haplotypes across the world in the last 100,000 years (Jobling and Tyler-Smith, 2003).

Another consequence of its inability to recombine is the accidental loss (genetic drift) of mutant-free Ys from the population, which can occur (just as for a surname) if the last possessors of a particularly fit Y by chance produce no sons. In a population of Y chromosomes that have suffered various amounts of damage, the best (least damaged) Y may be lost accidentally, and once gone it cannot be regenerated by recombination. This "Muller's ratchet" process can occur repeatedly, so that the second best, then the third best Ys are also lost until only heavily mutated Ys remain. Theory predicts that drift is especially damaging in small populations. This is a particular problem for the Y because its population is only 1/4 that of autosomes since everyone has two copies of each autosome, but only half the population has a single Y.

Thus it seems that genetic imperatives—higher variation, inefficient selection, and accidental loss—conspire against the Y chromosome, and it hardly surprising that it degrades rapidly. Perhaps what is surprising are the species in which the Y has conspicuously *not* degraded. Why have the emu W and the python W stayed more-or-less intact? Why do most genetic sex-determining fish and frogs *lack* differentiated sex chromosomes? Are they new systems, perhaps continually on the move like *Megaselia*? Or are there factors that can modify the rate of Y degeneration? I will examine these factors more specifically for mammalian sex chromosomes.

Rate of Y Degeneration

There is universal acceptance of the hypothesis that Y chromosomes degrade but widespread and vigorous debate about the rate at which this occurs and the prognosis for the human Y. Different models for degradation lead to different estimates of the time at which the human Y will completely run out of genes; estimates of extinction time for the human Y range from 125,000 years to infinity. Is degradation of the human Y slowing down or speeding up?

The average rate of loss of genes from the human Y is easily calculated from the numbers of genes lost from the human Y (from 1000 to 45) divided by the time over which this loss occurred ($\sim$300 MY). This comes to approximately 3.3/MY, and extrapolation of this linear loss leads to a predicted extinction time of about 14 MY (Figure 6A), down from the original estimate of 10 MY (Aitken and Graves, 2002) because the number of genes on the human X has been recalculated from 1600 to 1000.

However, it is very unlikely that the rate of loss is uniform over time. Gerrard and Filatov (2005) have pointed out that, as the target size decreases from 1000 to 500 to 200 genes, the rate might be expected to decay exponentially (Figure 6A). However, the target size argument is not this simple, as the male-specific region does not start with 1000 genes. In fact, when the proto-Y is created, the male-specific region contains only a single gene, so initially, at least, the target must *expand* as recombination is progressively restricted with time. Changes in target size will reflect the balance between recruitment into the MSY and gene loss from the MSY. This is hard to model, but initial increases of target size and later restrictions on target size seem likely to change the rate of degradation of the Y as a sinusoidal function (Figure 6A).

An important, if inscrutable, effect on the rate of Y degradation is the strength of positive selection. As degradation progresses, many genes acquire an important male-specific function (for instance *RBMY*, *SRY*), and these have been augmented, if only marginally, by the acquisition of autosomal male-advantage genes like *DAZ*. Some of these genes are clearly redundant, as seen by the spread of a Y chromosome haplotype deleted for several spermatogenesis gene families. However, others, such as *SRY*, have a unique function and must be now under positive selection. Presumably such unique genes become much more difficult to lose—yet the evidence of comparative genetics is that several have been

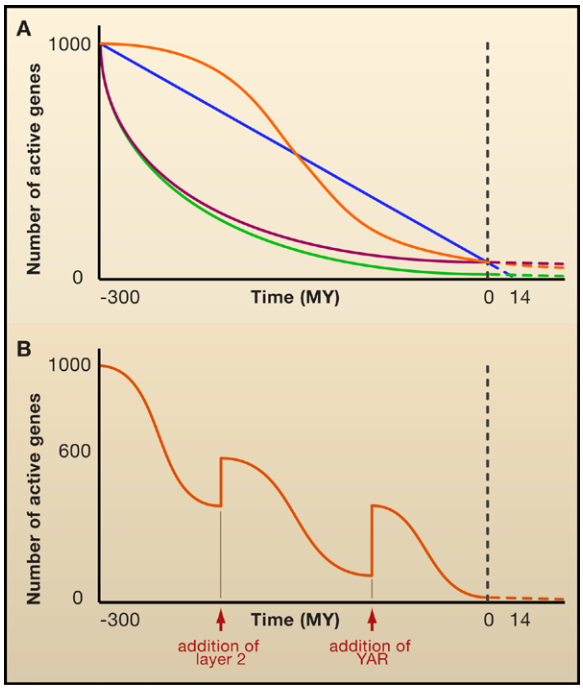

Figure 6. Kinetics for the Degradation of the Human Y
Possible trajectories for the degradation of the human Y chromosome from 310 MYA (when mammals diverged from reptiles) up to the present time (dashed vertical line), and prediction for extinction time.
(A) The rate of loss of active genes from the human Y assuming a constant rate (blue), an exponential decline (green), a target size that initially increases and then decreases (orange), or an exponential decline slowed down in its final stages by positive selection (purple).
(B) A more realistic picture of the rate of gene loss from the human Y, taking into consideration that at least three evolutionary blocks, the ancient conserved layer 1, the older layer 2, and the Y added region (YAR) were differentiated at different times, and each would have presented an initially small target size for degradation.

lost in one lineage or another. Their loss must require replacement of their function, perhaps by the X partner or another family member. For instance, in marsupials there are X and Y copies of the *ATRX*/*ATRY* gene, showing a somatic/gonad-specific division of labor. However, in placental mammals, *ATRY* has been lost and *ATRX* is expressed in somatic and gonadal tissues, suggesting that the gonadal function was resumed by *ATRX*.

Further complications in this analysis are posed by the different evolutionary layers of the human X and Y. The clustering of X-Y divergence times implies that degradation has not proceeded uniformly, either by a linear, exponential, or sinusoidal model. The three blocks from which the sex chromosomes were constructed each underwent decay as they were added, and the initial numbers of these genes, and the dates of their addition to the proto-Y, must be considered in plotting the decline and fall of the human Y. The oldest evolutionary layer (the conserved ancient X chromosome) contains about 500 genes, only four of which remain, and the next oldest (containing about 100 genes) has also been largely eliminated. However, the recently added layer 3 still retains about 38 of the original ~400 genes, as might be expected, since it has been degrading for only 100–180 MY. The kinetics of loss taking these factors into consideration is likely to have a wave form (Figure 6B). Obviously these considerations make it very difficult to make serious predictions of extinction time.

The rate of loss is also affected by other factors, many of which are specific to different lineages. For instance, some of the major degradative forces that have been outlined depend heavily on population size and mating structure. Generation time is an obvious factor since variation of Y sequences occurs during male germ cell formation, and this might explain why the rodent (particularly the mouse) Y chromosome seems to be more degraded and gene specialization more advanced than their orthologs on the human Y. For instance, *ZFY* is ubiquitously expressed in human males and its partner *ZFX* on the X is exempt from inactivation. In mouse, *Zfy* is testis specific and *Zfx* inactivated.

Population size also has a major effect on the probability that an active gene will be lost by drift, and the mating system has a big effect on the efficacy of the hitchhiker effect, which is driven by sperm competition. These factors can obviously differ greatly between even closely related species and, for instance, might explain why the chimpanzee Y chromosome seems to have degraded more rapidly over the last few million years than the human Y.

Thus many factors feed into equations describing the rate of degradation of the Y chromosome, and these make it difficult to predict how near to extinction the human Y really is. I challenge population and evolutionary geneticists to derive a meaningful model with predictive power. Essentially, the stochastic nature of many of the Y—major rearrangements and deletions on the negative side and acquisition of new male-advantage genes on the positive—means that it is at the mercy of chance events. It seems unlikely that the human Y has achieved a stable state. It would take substitution of function of only a few genes to render the human Y completely redundant and permit its complete loss (Figure 4B).

Extinction of the Y Chromosome

Calculations of the rate of loss of genes from the Y predict that, sooner or later, the Y will run out of genes altogether and disappear. This is not just a prophecy, but an observation in several different systems.

Again, *Drosophila* provides a spectacular example of the complete disappearance of the Y chromosome. The *D. melanogaster* Y appears to be completely nonhomologous to the X, and it was suggested that the original Y was depleted of active genes, or even completely lost, and replaced by a blob of heterochromatin (a B chromosome) that provided a pairing partner for the unpaired X. It then became the home of a number of genes recruited from autosomes that have a male-

specific function (Carvalho, 2002). However, this is just the beginning of the story of the comings and goings of *Drosophila* Y chromosomes. In one species group, the X fused with an autosome. This created another male-specific chromosome, and in *D. pseudoobscura*, the Y in turn was terminally degraded and lost (Carvalho and Clark, 2005). The added autosome was left as the sole Y and, true to form, is now degrading rapidly.

A number of rodents are also experimenting with modified X and Y chromosomes, in apparently quite independent attempts to modify the sex ratio to favor females (Fredga, 1983). The wood lemming has acquired a modified X* chromosome that suppresses the testis-determining action of the Y, so that X*Y, as well as XX and X*X*, animals are females. Surely this suppressor of *SRY* is the ultimate sexually antagonistic gene! In South America, a group of Akodont rodents have a modified Y chromosome that no longer bears a functional *SRY* gene, so that XY* as well as XX animals are females (Hoekstra and Edwards, 2000).

At the extreme are species that have dispensed with the Y chromosome, some from somatic cells, others completely. Some marsupials physically eliminate the Y from somatic cells during embryogenesis (Watson et al., 1999). Evidently the tiny marsupial Y chromosome contains no genes required for general functions in both sexes and is completely specialized for male function.

Two rodent groups have no Y, the mole vole (*Ellobius*) and the Japanese spinous country rat (*Tokudaia*). *Ellobius lutescens* is XO in both sexes, and *E. tancrei* is XX (with two identical X chromosomes derived by non-disjunction). The spinous country rat of Japan also has XO in both sexes (Arakawa et al., 2002). It was originally supposed that the testis-determining factor had moved to another location in the mole vole; however, careful screening of DNA from males and females demonstrated no trace of *Sry* or other Y markers (Just et al., 1995). This suggests that *Sry* has been replaced by a completely new sex-determining system in mole voles, and that fertility factors on the Y have also been substituted. In the country rat, no *Sry* was detected, but at least two other Y-borne genes (*Zfy* and *Tspy*) appear to be present in both sexes (Arakawa et al., 2002), suggesting that a region of the Y was transposed to the X or an autosome.

Do these systems represent the terminal stages of Y degradation, in which an impoverished Y can be lost with impunity—or, alternatively, the advent of a new system that has actively selected against the old Y? The movement of fertility genes from the Y in the country rat might have made its loss less drastic, for it is hard to see how loss of chromosome with more than one gene critical for sex and reproduction could be compensated for. Identifying new sex-determining genes, and therefore identifying nascent proto-XY (or a proto-ZW) systems in these Y-less species, will be very important for our further understanding of the process of sex chromosome evolution.

The mole vole and country rat demonstrate that extinction of the Y can and does occur in mammals as well as *Drosophila*. Why should the human Y be immune from further degradation and complete loss? Whether loss occurs in 125,000 or 14 or 140 MY, we should consider the possible consequences of extinction of the human Y.

Consequences of Extinction of the Y

Would loss of the Y chromosome and consequent loss of the *SRY* gene lead to the disappearance of males? If males became extinct, so would humans, because many maternally imprinted genes in the human genome are active only if they are derived from the male parent. The human race therefore must preserve males in order to continue reproducing.

Would loss of the Y lead to human extinction? Mole voles and country rats provide cheering evidence that a revolution in chromosomal sex need not herald the collapse of human reproduction. There are many ways in which a new sex-determining system could usurp the old without any changes being evident to humans (or voles). One possibility is that the *SRY* gene that triggers testis determination could be moved or copied onto a safer spot on an autosome, much as a copy of the *DMRT1* gene appears to have done in medaka fish. This would create a novel proto-Y and unleash a new round of sex chromosome differentiation.

Given that mutations in several genes downstream of *SRY* can cause sex reversal, there are opportunities for other genes in the pathway to take over as sex-determining master switch. For instance, increased dosage of *SOX9* or an upstream mutation can produce XX males (Qin and Bishop, 2005). An investigation of candidate genes in the mole vole has so far not revealed a new sex-determining gene (Baumstark et al., 2005), but a full genome screen could identify the gene that has taken over controlling *SOX9* and testis differentiation genes.

Thus the human race could carry on as if nothing had happened after the Y chromosome—in the long or the short term—becomes extinct.

REFERENCES

Aitken, R.J., and Graves, J.A.M. (2002). The future of sex. Nature *415*, 963.

Arakawa, Y., Nishida-Umehara, C., Matsuda, Y., Sutou, S., and Suzuki, H. (2002). X-chromosomal localization of mammalian Y-linked genes in two XO species of the Ryukyu spiny rat. Cytogenet. Genome Res. *99*, 303–309.

Baumstark, A., Hameister, H., Hakhverdyan, M., Bakloushinskaya, I., and Just, W. (2005). Characterization of Pisrt1/Foxl2 in Ellobius lutescens and exclusion as sex-determining genes. Mamm. Genome *16*, 281–289.

Carrel, L., and Willard, H.F. (2005). X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature *434*, 400–404.

Carvalho, A.B. (2002). Origin and evolution of the Drosophila Y chro-

mosome. Curr. Opin. Genet. Dev. *12*, 664–668.

Carvalho, A.B., and Clark, A.G. (2005). Y chromosome of *D. pseudoobscura* is not homologous to the ancestral *Drosophila*. Science *307*, 108–110.

Charlesworth, B., and Charlesworth, D. (2000). The degeneration of Y chromosomes. Philos Trans R Soc Lond Ser B *355*, 1563–1572.

Charlesworth, D., Charlesworth, B., and Marais, G. (2005). Steps in the evolution of heteromorphic sex chromosomes. Heredity *95*, 118–128.

Cooper, D.W., Johnston, P.G., Graves, J.A.M., and Watson, J.M. (1993). X-inactivation in marsupials and monotremes. Sem. Dev. Biol. *4*, 117–128.

Delbridge, M.L., Lingenfelter, P.A., Disteche, C.M., and Graves, J.A.M. (1999). The candidate spermatogenesis gene RBMY has a homologue on the human X chromosome. Nat. Genet. *22*, 223–224.

Delbridge, M.L., Longepied, G., Depetris, D., Mattei, M.-G., Disteche, C.M., Graves, J.A.M., and Mitchell, M.J. (2004). *TSPY*, the candidate gonadoblastoma gene on the human Y chromosome, has a widely expressed homologue on the X – implications for Y chromosome evolution. Chromosome Res. *12*, 345–356.

Dorus, S., Gilbert, S.L., Forster, M.L., Barndt, R.J., and Lahn, B.T. (2003). The CDY-related gene family: coordinated evolution in copy number, expression profile and proten sequence. Hum. Mol. Genet. *12*, 1643–1650.

Ellegren, H., and Fridolfsson, A.K. (1997). Male-driven evolution of DNA sequences in birds. Nat. Genet. *17*, 182–184.

Ezaz, T., Quinn, A., Miura, I., Sarre, S., Georges, A., and Graves, J.A.M. (2005). The dragon lizard *Pogona vitticeps* has ZZ/ZW microsex chromosomes. Chromosome Res. *13*, 763–776.

Foster, J.W., and Graves, J.A.M. (1994). An SRY-related sequence on the marsupial X chromosome: implications for the evolution of the mammalian testis-determining gene. Proc. Natl. Acad. Sci. USA *91*, 1927–1931.

Fredga, K. (1983). Aberrant sex chromosome mechanisms in mammals. Evolutionary aspects. Differentiation *23* Suppl., S23–S30.

Fridolfsson, A.K., Cheng, H., Copeland, N.G., Jenkins, N.A., Liu, H.C., Raudsepp, T., Woodage, T., Chowdhary, B., Halverson, J., and Ellegren, H. (1998). Evolution of the avian sex chromosomes from an ancestral pair of autosomes. Proc. Natl. Acad. Sci. USA *95*, 8147–8152.

Gerrard, D.T., and Filatov, D.A. (2005). Positive and negative selection on mammalian Y chromosomes. Mol. Biol. Evol. *22*, 1423–1432.

Glas, R., Graves, J.A.M., Toder, R., Ferguson-Smith, M., and O'Brien, P.C. (1999). Cross-species chromosome painting between human and marsupial directly demonstrates the ancient region of the mammalian X. Mamm. Genome *10*, 1115–1116.

Graves, J.A.M. (1995). The origin and function of the mammalian Y chromosome and Y-borne genes- an evolving understanding. Bioessays *17*, 311–320.

Graves, J.A.M. (2002). The rise and fall of *SRY*. Trends Genet. *18*, 259–264.

Graves, J.A.M., and Schmidt, M.M. (1992). Mammalian sex chromosomes - design or accident? Curr. Opin. Genet. Dev. *2*, 890–901.

Graves, J.A.M., and Shetty, S. (2001). Sex from W to Z – evolution of vertebrate sex chromosomes and sex determining genes. J. Exp. Zool. *281*, 472–481.

Graves, J.A., Disteche, C.M., and Toder, R. (1998). Gene dosage in the evolution and function of mammalian sex chromosomes. Cytogenet. Cell. Genet. *80*, 94–103.

Graves, J.A.M., Gecz, J., and Hameister, H. (2002). Evolution of the human X--a smart and sexy chromosome that controls speciation and development. Cytogenet. Genome Res. *99*, 141–145.

Grützner, F., Rens, W., Tsend-Ayush, E., El-Mogharbel, N., O'Brien, P.C., and Jones, R.C. (2004). In the platypus a meiotic chain of ten sex chromosomes shares genes with the bird Z and mammal X chromosomes. Nature *432*, 913–917.

Grützner, F., Ashley, T., Rowell, D.M., and Graves, J.A.M. (2006). How did the platypus get its sex chromosome chain? A comparison of meiotic multiples and sex chromosomes in plants and animals. Chromosoma, in press.

Hayman, D., and Sharp, P. (1981). Verification of the structure of the complex sex chromosome system in Lagorchestes conspicillatus Gould (Marsupialia:Mammalia). Chromosoma *83*, 263–274.

Heard, E. (2004). Recent advances in X-chromosome nactivation. Curr. Opin. Cell Biol. *16*, 247–255.

Hoekstra, H.E., and Edwards, S.V. (2000). Multiple origins of XY female mice (genus *Akodon*): phylogenetic and chromosomal evidence. Proc. R. Soc. Lond. B. Biol. Sci. *267*, 1825–1831.

Hughes, J.F., Skaletsky, H., Pyntikova, T., Minx, P.J., Graves, T., Rozen, S., Wilson, R.K., and Page, D.C. (2005). Conservation of Y-linked genes during human evolution revealed by comparative sequencing in chimpanzee. Nature *437*, 100–103.

Hurst, L.D. (1994). Embryonic growth and the evolution of the mammalilan Y chromosome. I. The Y as an attractor for selfish growth factors. Heredity *73*, 223–232.

Jegalian, K., and Page, D.C. (1998). A proposed path by which genes common to mammalian x and Y chromosomes evolve to become X inactivated. Nature *394*, 776–780.

Jobling, M.A., and Tyler-Smith, C. (2003). The human Y chromosome: an evolutionary marker comes of age. Nat. Rev. Genet. *4*, 598–612.

Just, W., Rau, W., Vogel, W., Akhverdian, M., Fredga, K., Graves, J.A.M., and Lyapunova, E. (1995). Absence of Sry in species of the vole Ellobius. Nat. Genet. *11*, 117–118.

Kohn, M., Kehrer-Sawatzki, H., Vogel, W., Graves, J.A., and Hameister, H. (2004). Wide genome comparisons reveal the origins of the human X chromosome. Trends Genet. *20*, 598–603.

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

Lahn, B., and Page, D.C. (1997). Functional coherence of the human Y chromosome. Science *278*, 675–680.

Lahn, B.T., and Page, D.C. (1999). Four evolutionary strata on the human X chromosome. Science *286*, 965–967.

Lahn, B.T., Pearson, N.M., and Jegalian, K. (2001). The human Y chromosome, in the light of evolution. Nat. Rev. Genet. *2*, 207–216.

Laumonnier, F., Ronce, N., Hamel, B.C., Thomas, P., Lespinasse, J., Raynaud, M., Paringaux, C., Van Bokhoven, H., Kalscheuer, V., Fryns, J.P., et al. (2002). Transcription factor SOX3 is involved in X-linked mental retardation with growth hormone deficiency. Am. J. Hum. Genet. *71*, 1450–1455.

Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B., Kamal, M., Clamp, M., Chang, J.L., Kulbokas, E.J., 3rd, Zody, M.C., et al. (2005). Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature *438*, 803–819.

Marais, G., and Galtier, N. (2003). Sex chromosomes: how X-Y recombination stops. Curr. Biol. *13*, R641–R643.

Makova, K.D., and Li, W.H. (2002). Strong male-driven evolution of DNA sequences in humans and apes. Nature *416*, 624–626.

McCarrey, J.R., and Thomas, K. (1987). Human testis-specific *PGK* gene lacks introns and possesses characteristics of a processed

gene. Nature *326*, 501–505.

Muller, H.J. (1914). A gene for the fourth chromosome of *Drosophila.* J. Exp. Zool. *17*, 325–336.

Nanda, I., Shan, Z., Schartl, M., Burt, D.W., Koehler, M., Nothwang, H., Grutzner, F., Paton, I.R., Windsor, D., Dunn, I., et al. (1999). 300 million years of conserved synteny between chicken Z and human chromosome 9. Nat. Genet. *21*, 258–259.

Ohno, S. (1967). Sex Chromosomes and Sex Linked Genes (New York: Springer-Verlag).

Orr, H.A., and Coyne, J.A. (1989). The genetics of postzygotic isolation in Drosophila virilis group. Genetics *121*, 527–537.

Page, D.C., Mosher, R., Simpson, E.M., Fisher, E.M., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A., and Brown, L.G. (1987). The sex-determining region of the human Y chromosome encodes a finger protein. Cell *51*, 1091–1104.

Park, D.J., Pask, A.J., Huynh, K., Harley, V.R., Renfree, M.B., and Graves, J.A. (2005). Characterisation of the marsupial-specific ATRY gene: Implication for the evolution of male-specific function. Gene *362*, 29–36.

Pask, A.J., Renfree, M.B., and Graves, J.A.M. (2000). The human sex-reversing *ATRX* gene has a homologue on the marsupial Y chromosome, *ATRY*: implications for the evolution of mammalian sex determination. Proc. Natl. Acad. Sci. USA *97*, 13198–13202.

Peichel, C.L., Ross, J.A., Matson, C.K., Dickson, M., Grimwood, J., and Schmutz, J. (2004). The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. Curr. Biol. *14*, 1416–1424.

Qin, Y., and Bishop, C.E. (2005). Sox9 is sufficient for functional testis development producing fertile male mice in the absence of Sry. Hum. Mol. Genet. *14*, 1221–1229.

Reinke, V. (2004). Sex and the genome. Nat. Genet. *36*, 548–549.

Repping, S., van Daalen, S.K., Korver, C.M., Brown, L.G., Marszalek, J.D., Gianotten, J., Oates, R.D., Silber, S., van der Veen, F., Page, D.C., and Rozen, S.A. (2004). Family of human Y chromosomes has dispersed throughout northern Eurasia despite a 1.8-Mb deletion in azoospermia factor c region. Genomics *83*, 1046–1052.

Rice, W.R. (1987a). The accumulation of sexually antagonistic genes as selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. Evolution Int. J. Org. Evolution *41*, 911–914.

Rice, W.R. (1987b). Genetic hitchhiking and the evolution of reduced genetic activity of the Y sex chromosome. Genetics *116*, 161–167.

Ross, M.T., Grafham, D.V., Coffey, A.J., Scherer, S., McLay, K., and Muzny, D. (2005). The DNA sequence of the human X chromosome. Nature *434*, 325–337.

Rozen, S., Skaletsky, H., Marzalek, J.D., Minx, P.J., Cordum, H.S., Waterston, R.H., Wilson, R.K., and Page, D.C. (2003). Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. Nature *423*, 873–876.

Saifi, G.M., and Chandra, H.S. (1999). An apparent excess of sex- and reproduction-related genes on the human X chromosome. Proc. R. Soc. Lond. B Biol. Sci. *266*, 203–209.

Sarre, S.D., Georges, A., and Quinn, A. (2004). The ends of a continuum: genetic and temperature-dependent sex determination in reptiles. Bioessays *26*, 639–645.

Saxena, R., Brown, L.G., Hawkins, T., Alagappan, R.K., Skaletsky, H., Reeve, M.P., Reijo, R., Rozen, S., Dinulos, M.B., Disteche, C.M., and Page, D.C. (1996). The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. Nat. Genet. *14*, 292–299.

Shetty, S., Griffin, D.K., and Graves, J.A.M. (1999). Comparative painting reveals strong chromosome homology over 80 million years of bird evolution. Chromosome Res. *7*, 289–295.

Sinclair, A.H., Berta, P., Palmer, M.S., Hawkins, J.R., Griffiths, B.L., Smith, M.J., Foster, J.W., Frischauf, A.M., Lovell-Badge, R., and Goodfellow, P.N. (1990). A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature *346*, 240–244.

Skaletsky, H., Kuroda-Kawaguchi, T., Minx, P.J., Cordum, H.S., Hillier, L., and Brown, L.G. (2003). The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature *423*, 825–837.

Sykes, B. (2003). Adam's Curse. A Future Without Men (London: Transworld Publishers).

Touré, A., Clemente, E.J., Ellis, P., Mahadevaiah, S.K., Ojarikre, O.A., Ball, P.A., Reynard, L., Loveland, K.L., Burgoyne, P.S., and Affara, N.A. (2005). Identification of novel Y chromosome encoded transcripts by testis transcriptome analysis of mice with deletions of the Y chromosome long arm. Genome Biol. *6*, R102.

Tsend-Ayush, E., Onnebo, S.M.N., Grützner, F., Delbridge, M.L., Lewis, R.S., O'Sullivan, L.A., Ward, A.C., and Graves, J.A.M. (2005). The *RBMX* gene is essential for brain development in zebrafish. Dev. Dyn. *234*, 682–688.

Traut, W., and Willhoeft, U. (1990). A jumping sex determining factor in the fly Megaselia scalaris. Chromosoma (Berl.) *99*, 407–412.

Veyrunes, F., Catalan, J., Sicard, B., Robinson, T.J., Duplantier, J.M., and Granjon, L. (2004). Autosome and sex chromosome diversity among the African pygmy mice, subgenus Nannomys (murinae; Mus). Chromosome Res. *12*, 369–382.

Wakefield, M.J., Keohane, A.M., Turner, B.M., and Graves, J.A.M. (1997). Histone deacetylation is an ancient component of mammallian X chromosome inactivation. Proc. Natl. Acad. Sci. USA *94*, 9665–9668.

Wang, P.J., Mccarrey, J.R., Yang, F. and Page, D.C. (2001). An abundance of X-linked genes expressed in spermatogonia. Nat. Genet. *27*, 422–426.

Waters, P., Duffy, B., Frost, C.J., Delbridge, M.L., and Graves, J.A.M. (2001). The human Y chromosome derives largely from a single autosomal region added 80-130 million years ago. Cytogenet. Cell Genet. *92*, 74–79.

Watson, C.M., Margan, S.H., and Johnston, P.G. (1999). Sex chromosome elimination in the bandicoot Isoodon macrourus using Y-linked markers. Cytogenet. Cell Genet. *81*, 54–59.

Wu, C.I., and Xu, E.Y. (2003). Sexual antagonism and X inactivation--the SAXI hypothesis. Trends Genet. *19*, 243–247.

Zechner, U., Wilda, M., Kehrer-Sawatzki, H., Vogel, W., Fundele, R., and Hameister, H. (2001). A high density of X-linked genes for general cognitive ability: a run-away process shaping human evolution? Trends Genet. *17*, 697–701.