

Did sex chromosome turnover promote divergence of the major mammal groups?

De novo sex chromosomes and drastic rearrangements may have posed reproductive barriers between monotremes, marsupials and placental mammals

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Comparative mapping and sequencing show that turnover of sex determining genes and chromosomes, and sex chromosome rearrangements, accompany speciation in many vertebrates. Here I review the evidence and propose that the evolution of therian mammals was precipitated by evolution of the male-determining *SRY* gene, defining a novel XY sex chromosome pair, and interposing a reproductive barrier with the ancestral population of synapsid reptiles 190 million years ago (MYA). Divergence was reinforced by multiple translocations in monotreme sex chromosomes, the first of which supplied a novel sex determining gene. A sex chromosome-autosome fusion may have separated eutherians (placental mammals) from marsupials 160 MYA. Another burst of sex chromosome change and speciation is occurring in rodents, precipitated by the degradation of the Y. And although primates have a more stable Y chromosome, it may be just a matter of time before the same fate overtakes our own lineage.

Keywords:

■ animal genomics; de novo sex-determining genes; hybrid incompatibility; mammal evolution; sex chromosome differentiation and degradation; sex chromosome rearrangement

Introduction: Chromosome change and speciation

Older literature is replete with suggestions that chromosome rearrangements – particularly highly disruptive translocations between chromosomes – induce hybrid sterility, and serve to separate incipient species [1].

Many examples, particularly in grasshoppers and other insects, were put

forward and discussed [2], as well as examples from reptiles [3]. Many species complexes, indeed, contain species with few phenotypic differences, but chromosome exchanges that make hybrids sterile [2]. The ideas were propounded that such changes occurred in an individual, were inherited by the offspring, and became fixed by mating between sibs or other relatives in small populations. Homozygotes could mate effectively only within the small group; matings between this group and the original population suffered from hybrid infertility.

However, evidence that chromosome rearrangement is important in speciation was fragmentary, and modelling has been less than convincing (reviewed [4]). Views on the mechanisms of speciation, therefore, focused on the Muller-Dobzhansky model, whereby accumulation of small mutations in populations that were physically separated promoted divergence [5, 6]. Decades of work, primarily on *Drosophila melanogaster*, identified such ‘speciation genes’, whose mutation was associated with hybrid incompatibility, and similar genes are now found in many other organisms [7]. Many alternative and complementary models have been produced to explain the processes by which one species becomes two. Also the major tenets of the hypothesis that chromosome rearrangement is critical in speciation do

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not receive strong support from the more detailed and incisive analysis permitted by newer molecular techniques [8]. Many examples have been studied in which hybrid incompatibility is not a serious barrier to gene flow. Indeed, the importance of chromosome rearrangements has been suggested to be more in the isolation of regions of low recombination which might harbour packages of genes that work in one environment but not another [9, 10].

The role of chromosome change in speciation was also problematical because it has such drastic effects. The argument was that such changes occurred in a single individual, and could not spread through the population because of the infertility of hybrids. Because speciation was reckoned to require a long time during which changes were polymorphic, it was difficult to imagine that such drastic changes could remain in the population long enough to become fixed, and were, therefore, thought to follow, rather than to induce speciation. Yet drastic chromosome changes do get fixed, if rarely. So there must be a route from a single individual change, through the spread, at least to two individuals of the opposite sex, to enable homozygosity and fixation.

Sex chromosome turnover and rearrangement may present a special case. Sex chromosomes are known to have major effects on speciation in terms of Haldane's Rule and the large X effect, explored in detail in *Drosophila* but evident also in mammals [11, 12]. New comparative genetic data document many vertebrates in which autosomes in one species have become sex chromosomes in another, as well as many rearrangements of sex chromosomes and autosomes. Molecular techniques have also documented the special properties of differentiated sex chromosomes in hemizygous expression and dosage compensation, and sex-biased gene content. Hybrids between individuals with different sex pairs are much more likely to suffer from infertility because of disruptions, not only to pairing and recombination, but also to sex determination and dosage compensation and to the disruption of the activity of fertility genes on sex chromosomes [13].

Here I argue that sex chromosome change, which is both more damaging

because of specialisation and degradation of sex chromosomes, but also more able to persist because incompatibility may be asymmetric (that is, has different effects in reciprocal crosses), precipitated divergence of the major mammal groups.

How are sex chromosomes rearranged or replaced?

In amniotes (mammals, birds and reptiles), the genome and the karyotype are amazingly conserved; there are few major chromosome rearrangements between an emu and a Galapagos tortoise, a snake and a chicken. Eutherian (placental) mammals are the glaring exception, showing a great variety of chromosome numbers and genome rearrangements; even the other two branches of mammals (marsupials and monotremes) have very conserved karyotypes [14].

However, even in taxa with extremely conserved karyotypes, sex chromosome turnover can take place when a novel gene on an autosome takes over the job of kick-starting gonad differentiation.

Fish provides numerous examples of sex chromosome turnover. For instance, in medaka and its close relatives with similar karyotypes, different chromosomes show sex linkage, as a result of the rapid evolution of different sex determining genes [15–17]. In sticklebacks, too, novel sex genes have rapidly arisen [18, 19], and in cichlid fish of Lake Victoria, rampant speciation is often accompanied by sex chromosome turnover [20]. In frogs, too, different sex determining genes and sex chromosome systems have arisen, and one species is polymorphic for XY and ZW systems [21–24].

In some reptile lineages, sex chromosome turnover is rare. For instance, all birds have a ZZ male:ZW female system and share the same Z chromosome, determining sex by means of dosage of a Z-borne gene *DMRT1* [25]. Snakes, with a different ZW pair, also appear to be very stable, at least in the two families (Colubridae and Viperidae) with highly degraded W chromosomes [26].

However, sex chromosome change is extremely frequent in other reptile families (e.g. lizards). One extra factor in

reptiles is the ability of many species to determine sex, not by sex chromosomes and genes, but by environmental temperature. There are phylogenies of closely related agamid lizards that reveal at least four flip-flops through genetic sex determination (GSD) and temperature sex determination (TSD), generating novel sex chromosomes [27]. Some reptile species have sex chromosome systems that can be overridden by temperature [28, 29]; these dual systems are extremely labile, able to change rapidly to TSD systems and even between ZW and XY [30, 31]. Similar GSD-TSD transitions may be common in some fish and may also facilitate changes in sex determination [32–34].

Much has been written about evolutionary drive to fill subtly different niches, but perhaps the triggers for speciation in some vertebrate groups include the inherent instability of the sex determining system.

Mammal sex genes and sex chromosomes are very stable

In comparison, mammals have a very stable sex determination system. Since they are homeothermic, GSD to TSD transitions are not an option, and the XY pair of therian mammals has differentiated to extremes that make YY individuals inviable.

There are three major groups of mammals; Eutheria (placental) and Metatheria (marsupials) together comprising subclass Theria, which diverged from the egg-laying monotremes (Prototheria) 190 million years ago (MYA) [35] (Fig. 1). All eutherians have the same XY chromosome pair, defined by a male-dominant testis-determining gene identified as *SRY* [36]. *SRY* activates a genetic pathway that promotes differentiation of a bipotential gonad into a testis in the embryo and suppresses ovarian development [37, 38].

The gene content of the X is virtually identical over even the most distantly related eutherians. The Y chromosome is a relic of the X that degraded to different extents, leaving tiny overlapping sets of active genes [38]. The male-specific region of the human Y retains only 27 active genes, though some are

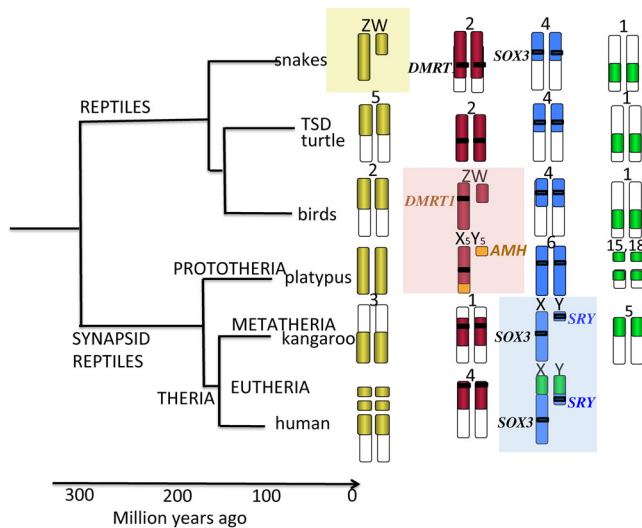


Figure 1. Relationships among higher vertebrate and their sex chromosomes. Placental mammals (infraclass Eutheria) diverged from marsupial mammals (Infraclass Metatheria) about 166 MYA [35]. These two groups comprise mammalian Subclass Theria, which diverged from Subclass Prototheria (monotreme mammals such as the platypus) 190 MYA. Ancestral synapsid (mammal-like) reptiles diverged from other reptile groups about 310 MYA. Comparative mapping demonstrates five orthologous segments that are sex chromosomes in one vertebrate lineage and autosomal in others. The yellow segment has become sex chromosomes in snakes (sex determining gene remains unknown). The red segment defined by *DMRT1* became sex determining in birds; this segment is represented on the sex chromosomes of platypus but the sex determining gene is *AMH*, translocated from another block (orange). The blue region defined by *SRY* (which evolved from *SOX3*) became sex chromosomes in mammals, and the green region fused with this ancient XY in eutherian mammals.

present in multiple copies (mostly inactive); cat and pig Y chromosomes have slightly more [39] and mice fewer [40]. Only two genes on the mouse Y are critical for a male phenotype and reproduction [41].

The evolution of *SRY* defined de novo XY sex chromosomes in therian mammals

In line with the accepted theory of sex chromosome evolution [42, 43], the evolution of *SRY* is credited with initiating the differentiation of the XY pair [38] (Fig. 2). *SRY* is thought to have evolved from *SOX3*, a highly conserved gene that lies on the X in mammals [44] and is expressed in the central nervous system and germcells, but not the somatic cells of the testis, where the sex determining message must be received [45]. Mis-expression of *SOX3* in the Sertoli cells causes sex reversal in humans and mice [46], suggesting that a change in tissue of expression,

perhaps by fusion with a gonad-specific promoter, created the male-dominant *SRY* gene that took over sex determination from the ancestral gene [47].

The emergence of a novel sex determining gene has been shown, in many systems, to drive the degradation of the chromosome on which it lies. In an ancient mammal, the acquisition of the dominant male determining *SRY* gene was, therefore, the kiss of death for the autosome that bore *SOX3*. Nearby genes were selected for a male-advantage function and the region became non-recombining to keep this male-specific package together [38]. Recruitment of more genes on the Y to a male-specific function extended this region progressively until only the terminal few megabases remained homologous to the X as the shared pseudoautosomal region.

Not only did the Y degrade, but both the X and Y rapidly specialised. Genes on the Y acquired male-specific functions such as in spermatogenesis. Meanwhile the X rapidly acquired sex and intelligence genes as a result of sexual antagonism and the hemizygous

state in males [38]. Dosage compensation evolved to silence one X in females, possibly after upregulation of genes on the X in both sexes [40].

Thus, a minor molecular accident – gene fusion and change of the transcription profile of *SOX3* – was all that was required to precipitate a major turnover of sex chromosomes in ancestral mammals.

Platypus sex chromosomes rewrote mammal chromosome evolution

When did this happen? And what was the sex determining system of the ancestor of all mammals which was replaced by the novel *SRY*-bearing chromosome pair in therians?

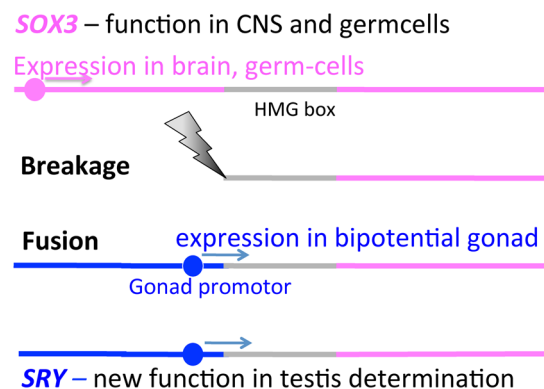
The standard approach to this question is to examine the sex chromosomes of outgroups. However, mammals and reptiles last shared a common ancestor 310 MYA, so the trail has gone a bit cold. Also, as we have seen, reptiles have an amazing array of different sex determining mechanisms, and exhibit frequent changes. It is difficult to deduce the sex determining system of a reptile ancestor, which might even have been environmental [48].

The basal group of mammals, the Prototheria, should provide some clues to the ancestral mammalian sex determining system. Monotremes (comprising two families, platypus and echidna) have multiple sex chromosomes [49]. The platypus has 10 sex chromosomes, five genetically distinct X and five Y. These form a chain at male meiosis which is held together by crossing over within nine pseudoautosomal regions. The five Xs segregate to one pole and the five Ys to the other [50].

The platypus orthologues of human X genes lie, not on the sex chromosomes, but on autosomes [51]. Mapping the genes on the five X and five Y chromosomes confirmed that platypus sex chromosomes have no homology to the therian X. But, surprisingly, they have extensive homology to the bird ZW sex chromosome pair [52].

Other gene blocks on the platypus sex chromosomes have homology to other chicken chromosomes, consistent

A Evolution of SRY



B Evolution of XY

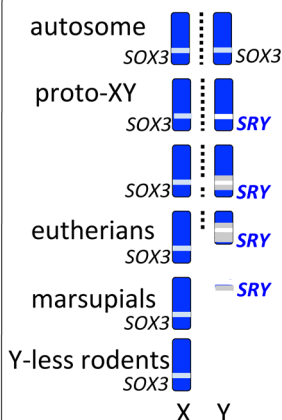


Figure 2. Evolution of *SRY* and the mammal XY sex chromosome pair. **A:** Evolution of *SRY* from an allele of autosomal *SOX3*. *SOX3* is highly conserved throughout vertebrates, with functions in brain and germcells, but is sex reversing when mis-expressed in the bipotential gonad. *SOX3* shares with *SRY* a moderately conserved HMG box (grey), involved with DNA binding and bending through a specific angle. We speculate that *SOX3* was truncated by breakage at the 5' end, and rejoining with a promoter that drove its expression into the bipotential gonad. **B:** Differentiation of the mammal X and Y chromosomes. Originally the X and Y were an ordinary pair of autosomes bearing *SOX3*. Mutation of one allele to a sex-determining *SRY* ensured that the chromosome that bore it became a male-specific Y. It degenerated rapidly as nearby genes acquired male-specific functions, and recombination (represented by dotted lines) in the region was suppressed to preserve a male-specific gene package. Within the region of low recombination, mutation, deletion amplification and insertion of repetitive sequence was rife, hastening progressive degeneration. Genes that acquired a male-specific function (grey) were retained. The XY of eutherian mammals retains a short pseudoautosomal segment that still recombines. However, the marsupial X and Y have lost all homology, and some rodents have completely lost the Y.

with the derivation of platypus chain by sequential translocations with autosomes [53]. Sequence comparisons between X-Y shared genes revealed that the large X_5 and tiny Y_5 that terminate the chain are the most diverged, and therefore the oldest members of the chain [54], suggesting that they represent the original mammalian sex chromosomes. X_5 is largely homologous to the bird Z [52].

The other monotreme family, the echidnas, also have a sex chromosome chain but it consists of only nine elements, with the tiny terminal Y_5 fused onto another Y [55]. Chromosome painting and gene mapping shows that four of the five XY pairs in the echidna chain are the same, but the fifth is represented by an autosome in platypus. Likewise, one of the platypus XY elements is represented by an autosome in echidnas. This implies that the three translocations in common occurred before platypus and echidna diverged,

probably about 50 MYA [56, 57], but one further translocation occurred independently in each of the two lineages at or after divergence, and involved different autosomes.

The identity of the monotreme sex determining gene has been controversial. Not surprisingly, monotremes lack *SRY*, and *SOX3* is autosomal [58, 59], lying with orthologues of other human X genes on chromosome 6. The bird sex determining gene *DMRT1* lies on an X chromosome in monotremes, so is unlikely to promote dosage-dependent determination in which two copies promote male development. There is no trace of a Y-borne *DMRT1* variant that could act as a male-determining gene. The most convincing candidate is an *AMH* orthologue that lies with syntenic markers on the tiny Y_5 chromosome [54]. This suggests that translocation of the ancestral sex pair with an autosome provided a novel sex gene in an ancestral monotreme. The other four Y

chromosomes are very degenerate; it will be interesting to discover whether they retain gene variants that have acquired functions in fertility.

We can date the evolution of *SRY* and the XY system of therian mammals from the known divergence dates of reptiles and mammal lineages (Fig. 1). Since birds and reptiles, and even monotremes do not share the therian XY, it must have evolved at or after the Prototheria-Theria divergence 190 MYA. And since marsupials and eutherians, which diverged 166 MYA, share *SRY* and the XY chromosome pair, *SRY* and the therian XY must have evolved in the 24 million year window between 190 and 166 million years ago.

Sex chromosome-autosome fusion accompanied evolution of eutherian mammals

Although the XY pair of marsupials and eutherians is monophyletic, there is an important difference. Comparative mapping of the marsupial orthologues of genes on the long arm and the pericentric region of the human X lie on the marsupial X, but more distal genes on the short arm of the human X map together on an autosome in marsupials [60].

This defines two ancient evolutionary blocks, the X conserved region XCR, which is shared by all therian mammals, and the X added region XAR, which is autosomal in marsupials.

Conserved and added regions YCR and YAR can be identified also on the human Y by mapping the X homologues of Y genes. The ancestor of *SRY* (*SOX3*) is one of the few Y genes that survive from the ancient XY; most human Y genes derive from the added region [61].

Regions orthologous to XCR and XAR are separate also in birds and other vertebrates [62], implying that they represent two separate ancient gene blocks that fused in eutherians. The addition of an autosomal region to the Y as well as to the X chromosome implies that fusion occurred within a large pseudoautosomal region when the XY pair was only partially differentiated.

Again we can date this fusion by comparing distantly related eutherians, humans and the basal afrotherians

(Fig. 1). The finding that genes on the human X all lie also on the elephant X chromosome implies that the fusion occurred before the eutherian radiation about 105 MYA [63]. And the finding that in elephants, XCR and XAR are separated by the centromere, suggests that the two ancestral blocks underwent centromere-centromere (Robertsonian) fusion [64].

Sex chromosome changes define the three major mammal groups

These data imply that three major sex chromosome changes separate the three major mammal lineages:

- de novo evolution of *SRY* and degeneration of the neo Y chromosome in a therian ancestor 190–166 MYA,
- three X-autosome translocations in monotremes 190–55 MYA, and a further independent translocation in platypus and echidna lineages,
- Robertsonian fusion of XY and autosome in eutherians 166–105 MYA.

We do not know how sex determination operated in the ancestor of all mammals, but it is clear that it did not involve *SRY*, whose ancestor *SOX3* is autosomal in monotremes as well as other vertebrates. The homology between monotreme and bird sex chromosomes initially suggested that the mammalian ancestor had a bird-like ZW system, driven by *DMRT1* dosage. However, this need not represent identity by descent, for a flatfish also has a ZW sex pair with homology to the bird ZW, and uses *DMRT1* dosage to determine sex [32]. *DMRT1* has also been pressed into service independently by spawning copies that became a dominant male-determiner in medaka [16, 65] or female determiner in *Xenopus* [66]; clearly this gene is simply good at sex determination and has been independently recruited in several lineages [67].

In order to explore specific consequences of a take-over by a de novo *SRY*-driven XY system, I shall posit that an ancient ZW system, similar to that in birds, prevailed in the ancestor of all mammals. I propose that *SRY* arose on a

ZZ background after the mis-expression of *SOX3* in the bipotential gonad, and became fixed in a small population, which could no longer effectively mate with the original population. This population gave rise to therian mammals.

Each of these changes would have been highly disruptive to hybrids. Matings between animals with a primitive bird-like ZW system and the de novo XY system could produce offspring with mixtures of sex chromosomes that are likely to be infertile or even inviable (Fig. 3). The degree of disruption would depend on the rapidity of sex chromosome differentiation and degradation. If Y chromosome change and dosage compensation were rapidly established, offspring of heterogametic XY males and ZW females would suffer from haploinsufficiency, exacerbated by specialisation of the X and the evolution of dosage compensation (Fig. 3b). If the de novo XY system maintained homomorphy for longer, the two systems might co-exist for many generations. Interestingly, the results of reciprocal crosses might differ; a cross between homogametic XX females and ZZ males might deliver some viable and fertile individuals (Fig. 3c), and thus might maintain a polymorphism for many generations.

The effects of translocations and fusions between sex chromosomes and autosomes are well known in mice and humans [68, 69]. Individuals heterozygous for translocation produce half balanced and half unbalanced gametes, a severe reproductive barrier between old and new systems. Interference with the dosage compensation system appears to contribute to infertility in mouse translocation hybrids [70].

It is also interesting to explore what might happen when a sex chromosome-autosome translocation takes place, as was the case in ancestral monotremes. Again, for argument's sake, I will propose that monotremes originally had a bird-like ZW system driven by dosage of *DMRT1*. The first translocation to this ancestral Z (probably in a ZZ male) added a region that contained the *AMH* gene, an important player in the sex determining network in reptiles, and the sex determining gene in some fish [16, 71]. I propose that *AMH* took over as a male-dominant sex determining gene, defining a neo-Y. A system of alternate segregation evolved to segregate multiple

X and Y chromosomes into X- and Y-bearing sperm. This change could have promoted or reinforced monotreme divergence, since translocation heterozygotes would produce unbalanced gametes, as well as having two, possibly antagonistic, sex determining genes. Subsequent translocations would have been less disruptive, since alternate anaphase segregation had already evolved; however, a fourth translocation, different in platypus and echidna, may have promoted divergence of families Ornithorhynchidae (platypus) and Tachyglossidae (echidnas).

Either change could have been first; de novo XY evolution may have precipitated divergence of Theria, leaving the ancient ZW system to be later salvaged by sex chromosome-autosome translocation in Prototheria. Alternatively an initial translocation may have been sufficient to drive apart the ancestors of Theria and Prototheria, with one lineage evolving alternate segregation, and the other soldiering on until it evolved a novel sex chromosome system.

In the therian lineage, a further major change, fusion of the XY with an autosome, may have promoted divergence of eutherian mammals from marsupials.

Thus the differences in sex chromosomes of the three major mammal groups would all be highly disruptive to hybrid matings. I propose here that these changes, rather than merely following the isolation of mammal populations, had a major role in precipitating divergence.

How does sex chromosome turnover occur?

This scenario for the sex chromosome-driven divergence of major mammal groups calls into question how sex chromosome turnover occurs, how it can be fixed in a population, and the selective forces promoting and opposing fixation. There is a catch-22 here; divergence depends on a reproductive barrier being posed by hybrid infertility, and the barrier is highest after the most drastic changes. However, the more drastic the change, the less likely it is to be fixed.

The problems of accounting for chromosome change have been debated

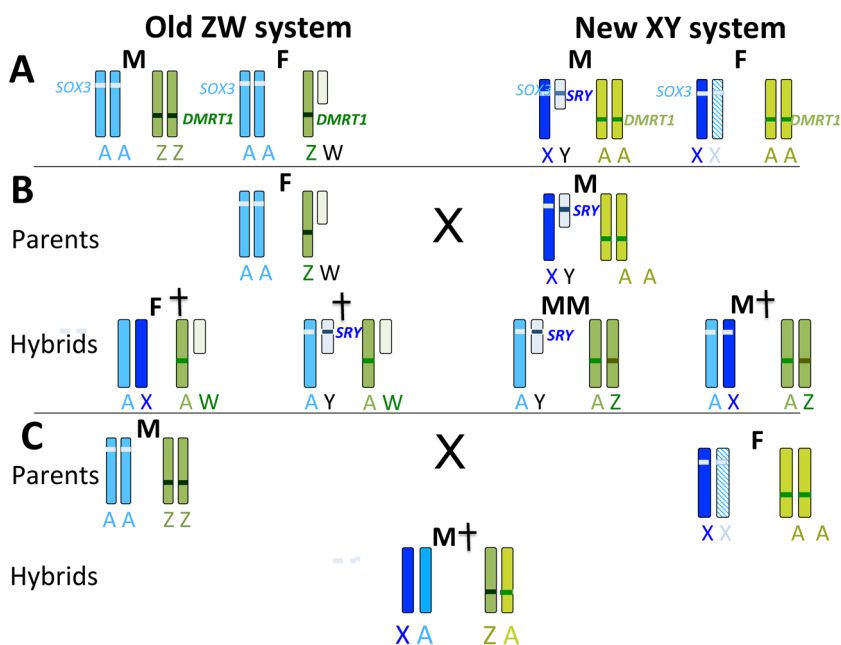


Figure 3. Interaction of ancient ZW and de novo XY sex determining systems. **A:** In the old ZW system, the male (M) has two Z chromosomes (green), bearing *DMRT1*. Females (F) have a single Z and a degenerate W. Dosage of *DMRT1* determines sex; two copies are required for male development (dark green). Some upregulation of Z genes occurs in females. An autosome (blue) bearing *SOX3* (light blue) is present in two copies in both sexes. In the new XY system, with the evolution of *SRY* (dark blue) from *SOX3*, the blue autosome has become a sex pair. Males have a single X and a degenerate Y bearing the male-dominant testis determining gene *SRY*; genes on single X are upregulated (darker blue). Females have two X chromosomes; one upregulated (darker blue) and one inactive (blue hatching). Gene content of the X has become specialised. The Z chromosome has become an autosome (A, lighter green), losing its dosage compensation and *DMRT1* is no longer sex determining (lighter green). **B:** Matings between heterogametic female (ZW) and heterogametic male (XY) produce hybrid offspring with four combinations of ZW and XY chromosomes, with predicted phenotypes as shown (male M, female F, dead †). Hybrids with two green chromosomes may be male with or without *SRY*. Only offspring with two blue chromosomes and a single green chromosome are likely to be female. Offspring with both a degenerate W and Y are likely to be inviable, and offspring with two blue chromosomes (an X and autosome) could suffer from dosage problems (too much product if the X is upregulated; too little if it is inactivated). **C:** Matings between homogametic male (ZZ) and homogametic female (XX) produces offspring with only one combination of sex chromosomes. With two *DMRT1* copies these are likely to be male; however, the combination of an X and the original autosome may not be viable if the X is upregulated or inactivated.

over decades. A chromosome rearrangement must occur, or a novel sex determining gene must arise, in a single individual. How can this spread in a population when it leads to infertility? Turnover of mammal sex chromosomes is equally difficult to explain, whether or not it promotes divergence.

However, sex chromosome turnover has occurred in mammals, though rarely. What needs to be explored, therefore, is how and why does it get fixed? Is there selection for the new system, and on what characteristic?

In examining how a novel sex chromosome can take over a population,

it is useful to distinguish between young sex chromosome systems and highly differentiated sex chromosomes.

Young sex chromosomes, such as those in many fish and some reptiles, show little differentiation. Thus the Y (or W) chromosome has not yet degraded to the extent that vital genes have been lost, nor has dosage compensation evolved to any great extent. This means that YY (or WW) individuals are likely to be viable, and a new sex gene on another chromosome may exist as a polymorphism for a considerable time. This seems to be the case for the Japanese wrinkled frog *Rana rugosa*, in

which XY and ZW systems co-exist in a hybrid zone where many different sex chromosome combinations occur [23].

In such systems, the original sex chromosomes can readily revert to their previous status as autosomes. This appears to have occurred in medaka and related fish, in which an ancestral sex determining gene on one chromosome was replaced with a copy of the *DMRT1* gene on a different chromosome [65]. This can occur more easily if the sex chromosomes have differentiated to only a minor extent; however, highly diverged sex chromosomes may survive as autosomes if the de novo system arises on either an XX female or a ZZ male background.

The extent to which replacement of highly differentiated sex chromosomes with a novel system poses a reproductive barrier between old and new sex systems will depend on the rapidity with which the novel system differentiates. Hybrids will be subfertile, producing unbalanced gametes and duplication-deficient offspring (Fig. 3). In addition, there is likely to be derangement of expression of genes on the X (or Z) that are dosage compensated.

How do new sex chromosomes become fixed?

However, it is more difficult to explain how such a drastic alteration in a single individual can become fixed in a population. The classic explanation is that random mutations or rearrangements occur by chance in small populations and can achieve homozygosity by inbreeding [1]. There is ample evidence that chromosome rearrangements may occur and be randomly fixed in small populations without being adaptive. For instance, many different chromosome fusions have become fixed in isolated populations of mice introduced to Mauritius only 700 years ago; these populations are now unable to hybridise [72]. This is far too short a time to envisage gradual accumulation of mutants in isolated populations that ultimately pose a reproductive barrier, and the most sensible conclusion is that non-adaptive chromosome change and drift has promoted speciation.

Although many authors have discounted the possibility that drastic chromosome change or sex chromosome turnover can occur and be fixed, we have two examples of recent turnover in groups of rodents that have replaced *SRY* and lost the entire Y chromosome.

The better characterised of these are species of the spiny rat *Tokudaia*, which live a precarious existence on islands of south Japan. Two species have XO males and females; they lack *SRY*, and other fertility genes on the mouse Y have been moved or replaced. There are mutations in the target gene *SOX9* [73], and a novel gene *CBX2* (Chromobox protein homologue 2) appears to have been elevated from its subservient role in gene regulation via chromatin remodelling to the head of the sex determining pathway [74].

Independently, loss of the Y and *SRY* has occurred in two species of the mole vole *Ellobius* [75], which presumably now determine sex by an as yet undiscovered gene on the X or autosomes. The loss of the *SRY* sex determining mechanism seems to have been accompanied by changes in its target gene *SOX9* that may have led to its upregulation in the absence of *SRY* [76].

Interestingly, the X chromosome in both these groups of Y-less rodents has not reverted to an autosomal state [77], perhaps because X-borne genes are upregulated so that two copies are deleterious.

Clues to how loss of the Y could have occurred in spiny rats are provided by a third species (Fig. 4b) which has a bizarre Y, containing more than 100 amplified copies of a mutationally damaged *SRY*. This suggests that the *SRY* gene of a common ancestor underwent sequence changes that weakened the binding of *SRY* protein to DNA, so amplification of this mutant gene was strongly selected to compensate for its poor DNA binding. A change of function of *CBX2* to activate the autosomal *SOX9* could then have occurred, and this more robust system could have rapidly replaced the compromised *SRY* sex determination system.

More generally, I propose that a terminally differentiated and mutated Y chromosome may specify a compromised sex determination system, so its replacement by a de novo gene on an autosome has a selective advantage. Specifically for mammals I suggest that an old system was nearing the end of its useful life,

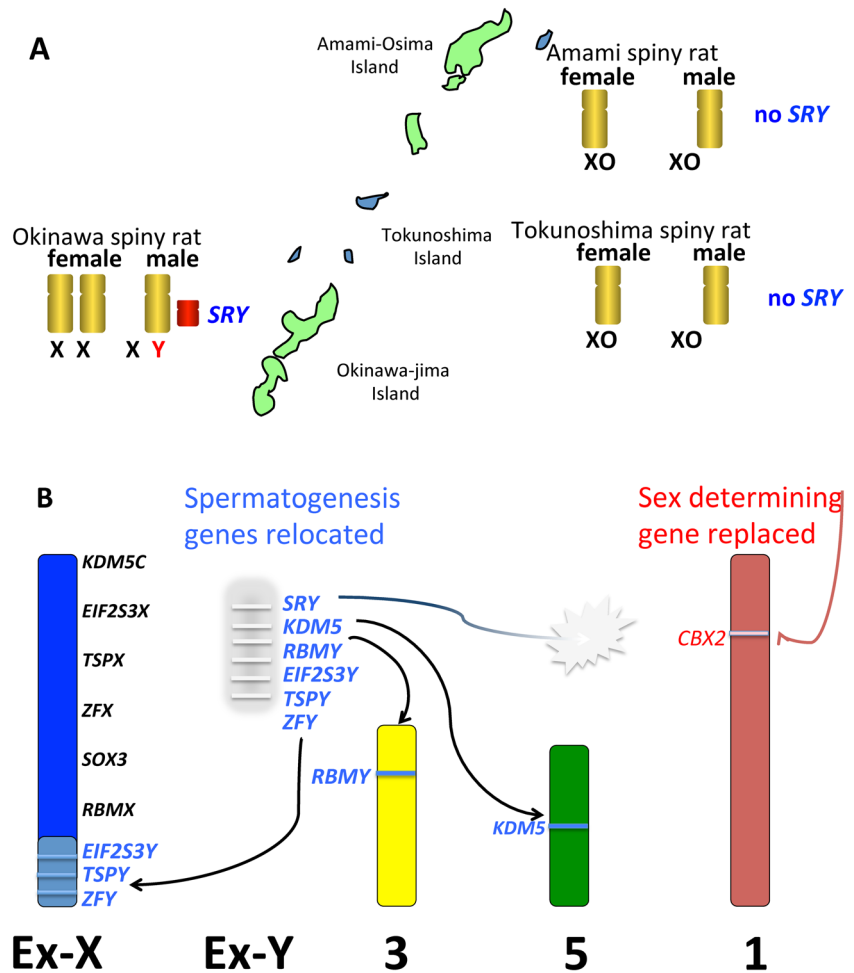


Figure 4. Turnover of sex chromosomes and speciation in the spiny rat. **A:** Three species of spiny rat (genus *Tokudaia*) live on islands in south Japan. The Amami spiny rat retains a Y chromosome but the Amami and Tokunoshima spiny rat have no Y and no *SRY* [80]. **B:** The process of loss of the Y. Fertility genes have been relocated to autosomes (chromosomes 3, 5) or the old X as shown, and the rest of the Y, bearing *SRY* (amplified and possibly inactive), was lost. A novel sex determining function was acquired by *CBX2* on chromosome 1.

with terminally differentiated sex chromosomes that bore a mutated sex determining gene, and were full of repetitive sequences and highly prone to rearrangement or deletion. Thus novel sex determining systems that arose independently in monotremes and therians could have been positively selected.

Can sex chromosome turnover promote divergence?

I have discussed how sex chromosome turnover, even drastic chromosome rearrangement and change from one differentiated sex pair to a novel system,

can and does occur, and can be fixed in a population, even in mammals, and can contribute to reproductive isolation.

But it is extremely difficult to show that chromosome change *causes* reproductive isolation, rather than simply following or being coincident with an accumulation of mutations that traditionally is credited with reproductive isolation. Obviously there are many divergence events that are decidedly *not* caused by chromosome change or sex chromosome turnover; witness the near identical gene content of X chromosomes across all eutherian lineages, and the completely stable karyotypes of 60 species of dasyurid marsupials.

However, it is remarkable that the major mammal groups are characterised

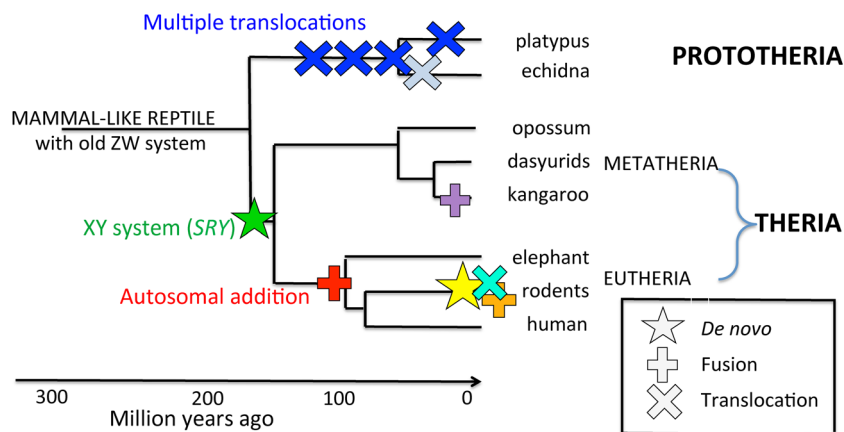


Figure 5. Major sex chromosome changes associated with divergence of mammal lineages. De novo evolution of *SRY* and the XY sex chromosome system (green star) occurred early in therian evolution and may have triggered divergence of Theria from Prototheria. At the same time, the ancient ZW system was bolstered by translocation with an autosome (blue cross), which may also have promoted divergence of Prototheria from Theria. Two further translocations occurred before divergence of platypus and echidna; different final translocations may have triggered divergence of Families Ornithorhynchidae (platypus) and Tachyglossidae (echidna). In the Theria, fusion of the XY with an autosome (denoted by +) occurred before eutherian radiation, and may have triggered divergence of Metatheria and Eutheria. Fusions are evident in some marsupial lineages, and many changes in sex determination (symbols in other colours) are observed in different rodent lineages which may signify a recent burst of sex chromosome turnover and speciation.

by sex chromosome rearrangement and turnover (Fig. 5), which are uncommon in mammals.

Remarkable though this coincidence is, it does not tell us that divergence was caused, or even promoted by, chromosome change. How might we distinguish the two hypotheses? Information about populations at deep divergence points such as those that separated the major three groups of mammals is inaccessible. Even where change has occurred over a brief evolutionary time, as in spiny rats, it remains possible that small island populations accumulated mutations that interposed reproductive barriers independently of sex chromosome turnover.

The most advantageous systems to analyse might be rodent groups that have undergone rapid divergence and seem to be undergoing sex chromosome turnover.

Is there a new round of mammalian sex chromosome turnover and speciation?

The mammal Y chromosome shows all the signs of extreme differentiation. The

mouse Y, particularly, seems to be near the end of its useful life. It contains only two genes essential for male phenotype, and these can be readily replaced; *SRY* by upregulating *SOX9*, and *EIF2S3Y* a sexually dimorphic transcription factor, by upregulating its X-borne partner *EIF2S3X* [78].

Many rodent species seem now to be experimenting with novel systems [79]. There are several examples of closely related rodent species with variant sex chromosomes. As well as the two genera that contain Y-less species discussed above, there are three species of Patagonian akodont mice that have evolved an inactive *SRY* gene, leading to *SRY*+ female mice [80]. Since other species in the same clade do not have *SRY*+ females, these changes were thought to be independent; however, they may have occurred after *SRY* was mutationally damaged, or relocated to part of the Y subject to epigenetic silencing in an ancestral animal. Wood lemmings and a pygmy mouse species have independently evolved variant X chromosomes that suppress *SRY* action, again leading to XY *SRY*+ females [79].

Pygmy mice are famous for rearrangements of sex chromosomes and autosomes [81]. There are X- and Y-autosome

fusions that differentiate closely related species, and some species have both, requiring segregation from a chain of four or even six chromosomes at male meiosis. This is reminiscent of the monotreme translocation complex – perhaps both were selected because they rescue an ancestral system that has become error-prone.

Nor are rodents the only taxon that is prone to sexual experimentation. Bats and shrews, too, show many sex chromosome-autosome fusions. Marsupials have XXY and XYY systems [82], and one fusion of a NOR-carrying autosomal regions [83] may have separated the kangaroo family from other marsupials 11 MYA.

Are rodents showing us the future for our own lineage? Human, indeed primate, sex chromosomes seem to be unusually stable [84], so the human Y may last far longer than the 4.6 MY predicted on the basis of linear decay, or even the more generous lifetime afforded by exponential decay and positive selection [38, 85]. But if primates survive for long enough, change will happen. Will new rounds of fusions rescue the dying Y as in monotremes? Or will novel genes evolve and define new sex chromosomes as in medaka fish and ancient therians? If it happens, will the novel sex chromosomes and sex determining systems promote hominid speciation?

Conclusion and outlook

New sex determining genes, and novel and rearranged sex chromosomes, characterise all three major branches of mammals. These types of sex chromosome turnover leads to infertility of hybrid offspring, because of unbalanced gametes and disruption of dosage compensation mechanisms.

How are such radical changes perpetuated and fixed in a population? Spread may have been facilitated by the unequal effects of reciprocal crosses between homogametic and heterogametic males and females. They may even have been selected for if the system they replaced was in a terminal stage of degradation.

Did these changes drive, or follow, divergence of mammal lineages? Each would provide a barrier to mating between

individuals with old and new sex chromosome systems. Chromosome speciation was discounted for decades in favour of hypotheses that populations first become isolated (physically or behaviorally), and diverged gradually by the accumulation of small mutations.

To distinguish between these two hypotheses, mammals with variant sex chromosome systems are particularly valuable. Several rodent lineages show recent chromosome rearrangement or turnover, with novel genes that substitute for – or repress – *SRY*. These novel systems could provide new insight about the role of sex chromosome turnover in speciation.

The other source of information might be species that recently entered a new niche, such as the mice in Mauritius in which chromosome change and speciation occurred too rapidly to allow for slow accumulation of mutants. Particularly revealing may be captive colonies of zebrafish, which independently lost their *W* and now rely on different genes in the sex determining pathway [86].

Complete genome sequencing of non-model vertebrates promoted by Genome 10K [87] may reveal many more systems that are in the process of sex chromosome change, and which we can exploit to determine under what conditions sex chromosome turnover leads to reproductive isolation, and how this translates into speciation.

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