Spring 2026 – Systems Biology of Reproduction
Lecture Outline – Sex Determination
Eric Nilsson – Biol 475/575
10:35-11:50 am, Tuesdays & Thursdays
January 27, 2026
Week 3

Sex Determination

- History
- Jost model of sexual differentiation
  - Chromosomal sex
  - Gonadal sex
  - Phenotypic sex
- Gonadal development systems
  - Cell biology
  - Required genes
- How does chromosomal sex dictate gonadal sex?
  - Molecular cloning of testis-determining factor(s) (e.g. SRY)
  - Interactions of SRY and SOX genes
  - X chromosome sex determining factor DSS/DAX
  - Interactions SRY, SOX, DAX, SF1, and DMRT
- How does gonadal sex dictate phenotypic sex?
  - Müllerian Inhibitory Substance (MIS)
  - Androgen induced male differentiation
- Abnormal sexual differentiation
  - New potential sex determination genes
- Mechanisms of sex determination in other species

Required Reading

Wilhelm and Pask (2018) Genetic Mechanisms of Sex Determination, in: Encyclopedia of Reproduction 2nd Ed. Vol 3, Pages 245-249.

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Genetic Mechanisms of Sex Determination

Dagmar Wilhelm and Andrew J Pask, The University of Melbourne, Parkville, VIC, Australia

Environmental sex determination (ESD) is believed to be the ancestral state, with genetic sex determination systems evolving later and independently in many lineages. ESD permits skewing of the sex ratio, which can maximize fitness in certain species or under a given set of environmental conditions (Adkins-Regan and Reeve, 2014). Conversely, genetic sex determination is controlled by one or more loci that are located on sex chromosomes and these mechanisms usually result in a stable 1:1 ratio of males-to-females in a population. Species with GSD are not affected by the external environment, which is important for thermo-regulated, viviparous species such as placental mammals, where sex determination and early sexual differentiation occurs in a controlled environment, in utero.

Sex Chromosome Systems

Sex chromosomes have evolved many times independently, but their evolutionary journey is surprisingly similar. Sex chromosomes develop from a pair of autosomes when one of the chromosomes gains a sex-determining locus. To keep the genes with the sex-specific function together, recombination becomes suppressed around this locus. The lack of recombination results in an accumulation of mutations and accelerated degradation of the sex-specific chromosome (for review: Ellegren, 2011; Graves, 2016). Therefore, the size difference between the sex-specific chromosome and its counterpart is an indication of the age of the sex chromosomes.

There are two main sex chromosome systems: XX/XY, in which the male is heterogametic, that is, carries two different sex chromosomes; and ZZ/ZW, where the female is heterogametic. Typical examples are humans with a XX females and XY males, and birds with ZW females and ZZ males (**Fig. 1**). However, there are variations to this basic model. For example, some sex chromosomes have evolved by translocations and/or fusions, such as the $Z_1Z_1Z_2Z_2$-male and Z_1Z_2W-female system in the Adélie penguin (Gunski et al., 2017), and the XY_1Y_2-male and XX-female system in the catfish *Harttia carvalhoi* (Centofante et al., 2006). One of the most complex sex chromosome systems exists in monotremes, such as the Echidna, with a $X_1X_2X_3X_4X_5/Y_1Y_2Y_3Y_4Y_5$-male and $X_1X_1X_2X_2X_3X_3X_4X_4X_5X_5$-female system where the sex chromosomes form translocation chains or rings during meiosis (Grutzner et al., 2004; Rens et al., 2004). In addition, some groups have appeared to have lost a sex chromosome. For example, in the vole *Microtus oregoni* females are XO, whereas males are XY (Ohno et al., 1966; Fredga, 1983). In contrast, in two *Ellobius* species, *Ellobius tancrei* and *Ellobius talpinus*, the Y chromosome is lost and both males and females are XX (Just et al., 1995, 2007). In the Japanese spiny rats *Tokudaia osimensis* and *Tokudaia tokunoshimensis*, as well as the mole vole *Ellobius lutescens*, both the Y and the second X chromosome are absent and all animals are XO (Arakawa et al., 2002; Just et al., 1995).

In addition to their differentiation, the gene content of the sex chromosomes also becomes unique over time. The eutherian Y chromosome harbors the sex-determining locus and contains only a handful of other genes, all of which function in either testis development, spermatogenesis or as basal transcriptional regulators (Bellott et al., 2014). The eutherian X chromosome shows an enrichment of genes involved in testis and brain function (Graves et al., 2002). This is due to altered selective pressures placed on sex chromosomes, stemming from their hemizygosity in males. Thus, in males, mutations on the X chromosome that confer a male selective advantage can be rapidly selected for even if they would be recessive in the heterozygous state. The Y chromosome

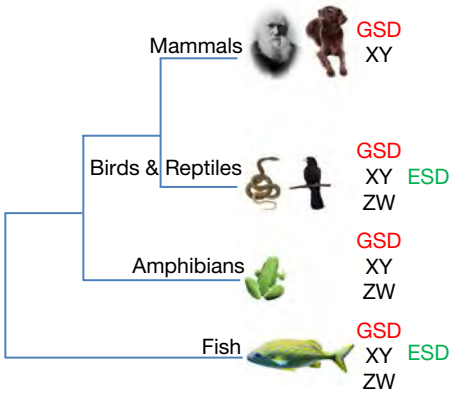

Fig. 1 Sex determination mechanisms in vertebrates. Vertebrate phylogeny showing the various methods of sex determination in each major lineage. Mammals have an exclusively GSD sex determination mechanism involving X and Y chromosome. Birds and reptiles show a broad range of mechanisms including GSD with exclusively ZW sex determination seen in birds but both XY and ZW systems and ESD seen in the reptiles. Amphibians appear to have largely GSD mechanisms with most species having cryptic sex chromosomes. Examples of amphibians with both XY and ZW systems have been described. Finally, fish have a broad array of mechanisms ranging from ESD to GSD including XY, ZW, and polygenic systems. Pictures obtained from https://pixabay.com/.

cannot contain any factors required for female function, so the only genes that remain are those that confer a male advantage or that are required to exist in two copies in both males and females (Whitworth and Pask, 2016). Similarly, the W chromosome in birds is enriched for female specific factors, owing to its hemizygosity in females (Moghadam et al., 2012).

Polygenic Systems of Sex Determination

In contrast to the XX/XY and ZZ/ZW system, in a polygenic sex determination system, multiple, independently segregating sex determining loci are present due to additional loci in the genome that can influence gonad development. This can arise through alterations of the sex chromosomes or of one of the autosomes, this can include, for example, the translocation of parts of the Y chromosome to an autosome, creating a so-called "neoY chromosome," resulting in a multiple sex chromosome system.

Polygenic sex determination mechanisms have been described in a various species of fish, insects, frogs, and even mammals. One of the first species for which a polygenic sex determining system has been identified is platyfish (*Xiphophorus maculatus*), which has a male-determining Y system as well as a female-determining W system. In this system males either are XY or YY, and females either XX, XW, or YW (Volff and Schartl, 2001). This means the hierarchy of these multiple sex chromosomes can be described as W > Y. However, this hierarchy is context dependent. For example, in the Western clawed frog *Xenopus tropicalis*, which also possesses three different sex chromosomes, W, Z, and Y, the hierarchy is Y > W. Therefore, males are either YZ, YW, or ZZ, and females are ZW or WW (Roco et al., 2015).

Examples of species in which a modification of a sex chromosome resulted in a polygenic sex determination mechanisms are the wood lemming, *Myopus schisticolor*, several species of the South African field mice (genus *Akodon*), and the African pygmy mouse, *Mus minutoides*. All three have an XY sex determination mechanism, but display both XX and XY females. However, the underlying modifications are different between the different species. In the wood lemming a mutation on the X chromosome, designated X*, most likely a structural rearrangement in the short arm of the X chromosome (Xp), results in the inactivation of the testis-determining factor on the Y chromosome. Therefore, three genotypically different females exist, XX, XX*, and X*Y. X*Y females only produce X*-containing oocytes, hence they give birth to daughters only (XX* and X*Y), resulting in approximately three to four-times more females than males in the population (Winking et al., 1981). Similarly, a chromosomal rearrangement of the X chromosome, however most likely a different one to that in the wood lemming, leading to an X* chromosome has been proposed in the African pygmy mouse. In contrast, in at least six species of *Akodon* the Y chromosome independently acquired a mutation resulting in a Y* and the complete failure to activate the male pathway (Bianchi and Contreras, 1967; Hoekstra and Edwards, 2000). Interestingly, sex reversed XY females in most mammalian species display greatly decreased fertility and fecundity (Marin and Baker, 1998). In contrast, in the species mentioned above, XY females are viable and fully fertile.

An example in which an autosome has undergone modifications is the house fly *Musca domestica*. The genome of the house fly consists of five autosomes and X and Y sex chromosomes. The Y chromosome harbors a male-determining factor M (Y^M). However, this factor can also be encoded on an autosome, A^M, or the X chromosome, X^M (Schmidt et al., 1997). In natural populations, male can carry one to several M factors (Hamm et al., 2014). In populations in which M is only on an autosome or the X chromosome, both males and females are XX (Franco et al., 1982; Hiroyoshi, 1964). The immediate downstream target of M is *Md-tra*, which is located on an autosome and exists in two variants, the wild-type allele *Md-tra* and a dominant allele, *Mda-tra*^{D}. While *Md-tra* is inhibited by M, *Mda-tra*^{D} is not and hence functions as a female-determining factor even in the presence of up to 3 M factors (Hediger et al., 1998, 2010).

Sex Determining Genes

While downstream genes in the sex differentiation regulatory cascade are conserved, the master sex determining gene that triggers sexual development shows, similar to the sex determination mechanisms, broad variations. The mode of action of this master sex determining gene can be either dosage sensitive, male- or female-dominant.

In mammals, the gene that initiates sexual development was discovered in 1990. The Y linked sex determining region on Y, or *SRY* gene was identified from analyses of human XY female and XX male patients (Sinclair et al., 1990). Experiments in mice revealed that it was both necessary and sufficient to drive testis development (Sinclair et al., 1990; Koopman et al., 1991). *SRY* encodes a transcription factor containing a high mobility group (HMG) DNA-binding domain, that gave the name to a whole family of transcription factor genes, the *Sox* (SRY-related HMG-box) family (Bowles et al., 2000). In contrast to *Sry*, which is only present in mammals, other *Sox* genes are conserved throughout the animal kingdom, including unicellular choanoflagellates (King et al., 2008). However, *Sry* is believed to have evolved from *Sox3* (**Fig. 2**), which is located on the X chromosome (Foster and Graves, 1994). SRY directly upregulates another HMG box gene, *Sox9* (Sekido and Lovell-Badge, 2008). SOX9 is, like SRY, necessary and sufficient to drive testis development in human and mice (Barrionuevo et al., 2006; Bishop et al., 2000; Chaboissier et al., 2004; Foster et al., 1994). As the only conserved domain between the two factors is the HMG domain, it has been suggested that other SOX proteins could function as male-determining factors. Indeed, ectopic expression of either SOX3 or SOX10 in human and mice result in testis development in XX individuals (Polanco et al., 2010; Sutton et al., 2011).

When *Sry* is not present or not functional, an ovary will form and therefore female development will occur. Hence, the female pathway has been seen as the default pathway. Nevertheless, there also is an active process driving ovarian differentiation, and while in mouse no ovarian counterpart for *Sry* has been identified, an ovarian-determining gene exists, for example in goat. Deletion of the

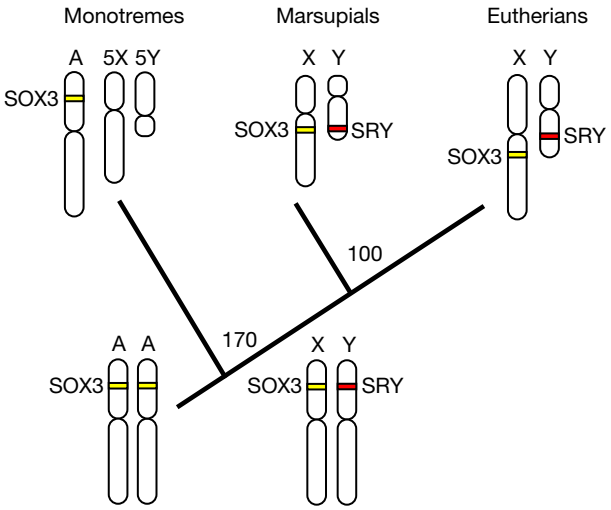

Fig. 2 Mammalian sex chromosome evolution. The sex chromosomes in all species originate from a homomorphic autosomal pair. One gene acquires the ability to determine sex. In mammals, this was the evolution of the *SRY* gene from its X-linked orthologue *SOX3*. Once the sex determination gene has been specified, recombination become restricted around this gene to prevent its cross over onto its chromosome pair. This restricted recombination leads to the accumulation of mutations, deletions and duplications causing the eventual evolution of heteromorphic sex chromosomes. The sex chromosomes also accrue a unique gene content owing to their sex specific distributions. A, Autosome, X, X-chromosome, Y, Y-chromosome. Numbers indicate the time of divergence in millions of years.

gene encoding the forkhead transcription factor FOXL2 results in testes development instead of ovaries, and therefore female-to-male sex reversal (Boulanger et al., 2014). Similarly, mutation of R-spondin 1 (*RSPO1*) in human can lead to complete female-to-male sex reversal in the absence of *SRY* (Parma et al., 2006). Mice with a null mutation in either *Foxl2* or *Rspo1* "only" display premature ovarian failure and partial sex reversal respectively (Uda et al., 2004; Chassot et al., 2008; Tomizuka et al., 2008), demonstrating clear differences between different mammalian species.

The identification of *SRY* as the male-determining gene in mammals triggered intensive research into the evolution of sex determining genes. It came to a surprise that no other vertebrate has the *SRY* gene. Instead, the first non-mammalian master sex determining gene that was identified in the Japanese rice fish medaka (*Oryzias latipes*) was *Dmy*/*Dmrt1bY* (DM domain gene on the Y chromosome/doublesex and mab-3 related transcription factor 1b on the Y chromosome) (Matsuda et al., 2002; Nanda et al., 2002). Interestingly, other members of the *Dmrt* gene family were independently recruited as master sex determining genes in other species. These include *Dmrt1* on the Z chromosome in birds, which confers male development using dosage sensitive mechanism (Smith et al., 2009), and *DM-W*, a truncated copy of *Dmrt1* on the W chromosome in the African clawed frog *Xenopus laevis*, which is a female-dominant gene driving ovarian development (Yoshimoto et al., 2008). This relatively widespread distribution of DM genes as master sex determining genes resulted in the suggestions that these could be the equivalent to the mammalian *Sry*. However, further analysis of the *Dmy*/*Dmrt1bY* in fish uncovered that it is absent in all other fish species studied (Kondo et al., 2003). It became clear that teleost fish not only represent nearly half of all extant vertebrates, but also display one of the widest variety of sex determination mechanisms, including a broad diversity of master sex determining genes. In addition to *Dmy*/*Dmrt1bY* in medaka, four other promising candidates have been identified to function as the trigger for sex differentiation. These include *amhy* (antiMüllerian hormone on the Y chromosome) in the Patagonian pejerrey *Odontesthes hatcheri*, *amhr2* (antiMüllerian hormone receptor 2) in the pufferfish *Takifugu rubripes*, *gsdf* (gonadal soma-derived growth factor) in *Oryzias luzonensis*, a species related to medaka, and *sdY* (sexually dimorphic on the Y chromosome) in the rainbow trout *Oncorynchus mykiss* and most other salmonids (Hattori et al., 2012; Kamiya et al., 2012, Myosho et al., 2012; Yano et al., 2012). Interestingly, none of these four genes encode transcription factors. Instead, two of these genes, *amhy* and *gsdf*, encode for growth factors, one, *Amhr2*, for a receptor, and the last one, *sdY*, for a protein that has homology to interferon regulatory factor 9 (IRF9), involved in SMAD signaling triggered by interferons. This demonstrated that a master sex determining gene does not have to encode a transcription factor to trigger sex differentiation.

Genetic Systems That Can Be Overruled by the Environment

Several groups of reptiles and fish show rapid evolutionary transitions between GSD and ESD mechanisms (**Fig. 1**; Quinn et al., 2011). Such plasticity in sex determination mechanisms can only occur in species with poorly differentiated sex chromosomes where essential genetic elements have not been lost to one of the sexes. Adding another layer of complexity, some species appear to have both GSD and ESD mechanisms operating concurrently, perhaps representing species at the transition from one mechanism to the other. One such species with both ESD and GSD is the European sea bass (*Dicentrarchus labrax* L.). While genetic mechanisms play a major role in sex determination in this species, elevated temperatures during early development result in masculinization of

fish that would develop into females at standard temperatures (Diaz and Piferrer, 2015). Similarly, in the Australian central bearded dragon lizard (*Pogona vitticeps*), which has a ZZ/ZW GSD system, elevated temperatures during development results in sex reversed genotypic males (ZZ) to phenotypic females (Quinn et al., 2007). Thus, in both cases, temperature can override the gene(s) involved in primary sex determination enabling skewed sex ratios under certain conditions.

Conclusions

From the studies mentioned above, it is clear that the sex determination switch is highly variable across the vertebrates and even between closely related species. In contrast, the underlying mechanisms which then direct the gonadal somatic cells towards either a male or female fate remain highly conserved. This begs the question of *why sex determination mechanisms are so variable?* It is clear that ESD mechanisms can increase fitness for some species where a certain temperature or skewing of the sex ratio leads to increased survivability. But it remains unclear why species with GSD would evolve such a variety of mechanisms and so rapidly transition from one gene driven system to another. This is especially puzzling given that determining sex correctly is arguably the single most important developmental trait in conferring fitness. Thus, we would predict that sex determination genes should be one of the most highly conserved aspects in our genomes.

Defining the mechanisms of sex determination, especially in species where it is rapidly evolving, is going to be critical for our understanding of why sex is so variable. The tractability of next-generation sequencing is likely to have a large impact on our understanding of vertebrate GSD mechanisms over the coming decade. It is now possible to sequence entire genomes from males and females in a population with no discernible sex chromosomes and simply compare the sexes to identify the genes which might be triggering the sex determination cascade. Such approaches can also be coupled with transcriptome sequencing of the developing gonad to identify not only the sex determination switch genes, but also the downstream genes and pathways activated in early sex fate choices across disparate species.

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Vertebrate sex determination: evolutionary plasticity of a fundamental switch

Blanche Capel

Abstract | The discovery of the *Sry* gene in 1990 triggered a revolution in our understanding of sex determination. More recently, advances in non-model organisms have been fuelled by the rapid evolution of affordable genome and transcriptome technologies. This Review considers the unusual plasticity in the bipotential system of sex determination and some of the diverse mechanisms that have evolved to control this critical developmental decision, including strong genetic pathways, environmental influences and epigenetic regulation. Ideas emerging from model and non-model organisms that suggest that sex determination operates as an antagonistic network with the emergent property of bistability are discussed.

Bipotential primordia
Primordial tissue that can take one of two fates.

Gonochoristic
Animals having two distinct sexes.

Primary sex determination
Based on the Jost paradigm: the decision within the gonad to initiate differentiation as a testis or an ovary.

Department of Cell Biology, 452 Nanaline Duke, 307 Research Drive, Duke University Medical Center, Durham, North Carolina 27710, USA.
blanche.capel@duke.edu

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Sex determination refers to the process by which a sexually reproducing organism initiates differentiation as a male or female. The mechanisms of sex determination are remarkably variable among organisms despite their critical importance for sexual reproduction and the survival of a species. This variability is in stark contrast to most other developmental processes, such as the formation of the body axis and the specification of the eye, which are highly conserved among species and are regulated by the same upstream gene networks. No single gene initiates sex determination in all species. Even when some of the same players participate in multiple species, they are often expressed in a different order. Moreover, among vertebrates, different cell types in the gonad can initiate the process of sex determination, with somatic cells driving the process in mammals and germ cells co-opting the driver's seat in many fish. What properties of the system accommodate such plasticity? How is this highly variable system suited to achieve reproductive fitness?

A unique characteristic of the reproductive system (as opposed to other organ systems) is that its anatomical components arise from bipotential primordia. This is true for species as distant as *Drosophila melanogaster* and *Mus musculus* and applies to both the gonad, which can develop as either a testis or an ovary, and the primordia for the genitalia, which follow a male or female developmental program. Independent primordia for both male and female sex ducts (in species where they exist) are present in the early embryo, but only one develops to channel gametes from the gonad to the outside world. This means that each embryo arises with the full potential to differentiate as either sex. In gonochoristic species, the business of sex determination is to activate one of the two developmental pathways and shut down the other.

Much of our understanding of how sex determination works in vertebrates comes from a paradigm established by the heroic experiments of Alfred Jost at the end of World War II. Working with rabbits, Jost developed a surgical method of removing the gonads from developing embryos and returning operated embryos to the uterus to complete development. Jost discovered that removal of the gonads from all embryos at mid-gestation led to the exclusive development of rabbits with female morphological sex characteristics. These experiments proved that (at least in rabbits) development of a phenotypic female does not require a gonad, but development of a phenotypic male does. From these experiments, Jost concluded that primary sex determination involves the decision to initiate testis or ovary development, which in turn leads to the production of substances that control the development of the sex ducts and genitalia[1]. He proceeded to show that the developing testis produces two critical substances that control sex determination[2]. The first is testosterone and its derivatives, which support the development of the male reproductive ducts (the epididymis and the vas deferens) and the male genitalia. The second substance, identified later as anti-Müllerian hormone (AMH)[3], controls the degeneration of the female duct primordia (which would otherwise give rise to the oviduct and uterus). Similarly, the primordium for the external genitalia is identical in all embryos but differentiates as male genitalia in the

Psychological sex
'Brain sex', inclusive of the gender with which an individual identifies and partner preference.

Gonadal sex determination
The decision to differentiate as a testis or ovary, referred to as 'primary sex determination' based on the Jost paradigm. However, evidence for sexual dimorphism before gonadal sex determination in many species suggests this term is more appropriate.

Genetic sex determination
(GSD). Sex determination that is driven by a gene or chromosomal difference between the sexes.

Environmental sex determination
(ESD). Sex determination driven by effects of the environment, which can include temperature, toxicants, population density, nutrients, hormones and behavioural cues.

Heteromorphic sex chromosomes
Sex chromosomes that are morphologically distinguishable.

presence of dihydrotestosterone[4] or as female genitalia in its absence. Compared with our understanding of how the sex organs develop, we know much less about how sex determination acts in the brain to establish morphological differences and psychological sex, encompassing gender identity and partner preference (BOX 1).

Although it is now clear that the gonad does not control all aspects of sexually dimorphic development in mammals or other vertebrates[5], because of its dominance over the most obvious sexually dimorphic characteristics, the central question in the field has been, "What initiates the differentiation of the gonad as a testis or ovary?" The control of gonadal sex determination is remarkably diverse[6]. Sex-determining mechanisms do not tend to cluster but are randomly dispersed throughout the vertebrate phylogenetic tree, suggesting that these mechanisms have evolved repeatedly[7]. Furthermore, closely related species depend on different mechanisms, suggesting that there is a low barrier to transitions between systems. Species have traditionally been classified as being governed by genetic sex determination (GSD) or environmental sex determination (ESD). However, many vertebrate species have been identified in which both GSD and ESD mechanisms operate simultaneously in response to a continuum of heritable and environmental factors[8,9].

What are the properties that allow the system to operate in the absence of consistent upstream regulators and with such extraordinary plasticity? Is there a common underlying pathway that controls differentiation of the testis or ovary? Are there evolutionary advantages to having sex determination systems that are not strongly hardwired? Despite the variability in the system, some unifying principles have emerged. This Review will focus primarily on vertebrates and uses mammals, one of the best genetic models, as an anchor point. I consider the inherent plasticity in the system, which is best illustrated in reptiles and fish, and some of the diverse mechanisms that have evolved to control the critical developmental decision of sex determination, including strong genetic pathways, environmental influences and epigenetic regulation. Lastly, I take a more global perspective to consider the idea that sex determination operates as a network with the emergent property of bistability.

Box 1 | **It is unclear how psychological sex is established**

The question of how psychological sex, including gender role and partner preference, is established is unclear[148]. Whereas the broad outlines of sexually dimorphic differentiation are worked out for the gonads, the sex ducts and the genitalia, this is not true for the brain, where it is possible that many surprises are in store. Like the genitalia and sex ducts, brain development was traditionally thought to depend on the hormone environment. However, sex-determining region Y (*Sry*) is expressed in some regions of the brain[149] and could have a direct influence on masculinization, independent of its influence on gonad development. In addition, the presence of XX or XY sex chromosomes may have a direct influence on brain development and some behavioural patterns[58,150]. Studies of psychological sex in patients with disorders of sexual development are helping to determine the weight of the hormonal and genetic factors that guide sex differences in brain development and behaviour patterns[151]. Investigations of specific features of neuronal development, such as the vomeronasal system, are also providing some answers about the pathways that underpin gender identity, mate selection and maternal behaviours (REF. 152 and references therein).

In the red-eared slider turtle, incubation at male- or female-producing temperatures leads to strong differences in brain development before the gonad forms[130]. This suggests that, at least in turtles, male or female brain development is independent of testis and ovary determination, as has been suspected for other reptiles[153]. This is a very interesting question in sex-reversing fish, where both gonad and gender identity can be reversed in adult life. In these cases, gonadal changes can occur without alteration of the behavioural phenotype, and behavioural changes frequently occur before gonadal changes, supporting the idea that their regulation is independent[103]. Further investigation will be required to discover how tightly gender identity and sexual phenotype are linked.

Most mammals depend on an XY system

In mammals, heteromorphic sex chromosomes (XY) have evolved. The Y chromosome carries the sex determining region Y (*Sry*) gene, which encodes a transcription factor that initiates testis development in the bipotential gonad[10,11]. *Sry* is a member of the SRY-box (Sox) gene family. Other members of this family — including the target of SRY, *Sox9* (REFS 12,13), as well as *Sox10* (REF. 14) and *Sox3* (REF. 15) (the evolutionary ancestor of *Sry*) — can substitute for *Sry* if expressed at the right time and place, suggesting that any Sox gene can activate the male cascade. Models for the evolution of *Sry* propose that an allelic change in the promoter or enhancer region of *Sox3* drove expression in the somatic cells of the early gonad, which activated testis development and led to the emergence of a pair of XY sex chromosomes[16]. Once a chromosome acquires a gene that promotes one sex or the other, it tends to accumulate other genes that increase the reproductive fitness of that sex[17]. For example, the Y chromosome also carries multicopy genes that are involved in optimizing spermatogenesis[18–20], thus favouring retention and transmission of the Y chromosome in males. Based on the infrequent transitions between sex-determining systems among mammals, this system appears to be self-reinforcing and stable.

We know the most about how sex determination works in mice. At the bipotential stage, the transcriptomes of cells in both the XX and XY gonad are nearly identical and biased towards a female fate[21] (FIG. 1a). An analysis in XY gonads revealed a poised state in which a male and a female sub-network are operating simultaneously[22] (FIG. 1b). In individuals with a Y chromosome, expression of SRY in the somatic supporting cell lineage leads to activation of its immediate downstream target *Sox9*. SOX9 acts in a feedforward loop with fibroblast growth factor 9 (FGF9), whose primary role is to repress Wnt family member 4 (*Wnt4*) and the female pathway (FIG. 1c). Loss of *Fgf9* leads to upregulation of *Wnt4* and causes sex reversal to female[23,24]. However, if *Wnt4* is simultaneously deleted, the pathway reverts to male[25]. Similar antagonistic relationships have been reported between *Sox9* and other members of the Wnt pathway, including R-spondin 1 (*Rspo1*) and catenin beta 1 (*Ctnnb1*)[26,27].

The current mammalian XY system with *Sry* at the top of the cascade evolved between 166 and 148 million years ago and has remained stable in most mammals studied. However, several exceptions are known. Both the spiny rat *Tokudaia osimensis* and the mole vole *Ellobius lutescens* have lost their Y chromosome and the

mammalian sex-determining gene *Sry*[28–30]. Both males and females are XO[31]. It has been proposed that ETS translocation variant 2 (ETV2; also known as ER71) has assumed the role of activating *Sox9* in the absence of *Sry*. Why this is not the case in XO animals that develop as females is not yet explained[32,33] but could be due to segregating allelic variants that do or do not have activity. By contrast, in at least nine species of *Akodon* South American grass mice, females can be either XX or XY* (where * designates an unknown change in the Y chromosome, defined functionally by sex-reversal)[34–37]. In *Akodon azarae*, although the *Sry* gene is present with no apparent mutations within the coding region, delayed or deficient expression levels due to epigenetic modifications are responsible for sex reversal in XY* females[34,38]. In this species, Y chromosomes that escape epigenetic silencing give rise to XY males. In a close relative of the house mouse, the African pygmy mouse *Mus minutoides*, many unusual sex chromosome translocations have been identified[39]. A high proportion of XY females harbour a rearranged X chromosome (X*) that is capable of triggering a normal ovary and female phenotype in X*Y animals[40]. The gene responsible for the feminizing influence of X* has not been identified. Theoretically, X*Y females should have severely reduced fertility due to meiotic defects and to the production of ¼ nonviable YY offspring when breeding with normal XY males, but X*Y females show more aggressive behaviour and (surprisingly) have higher reproductive output than either XX or XX* animals, which probably explains the maintenance of this system[41,42].

These cases highlight the resilience of the basic underlying system. The role of *Sry* is to tip the balance of antagonistic male and female networks towards a male fate. Once a male bias exists, it is amplified by FGF9 signalling[43] and additional signalling pathways, including desert hedgehog (*Dhh*)[44,45], prostaglandin D2 synthase (*Ptgds*)[46] and platelet-derived growth factor (PDGF)[47,48], that recruit other cells in the gonad to the male fate. Downregulation of genes associated with the female pathway in XY gonads is fundamental to commitment to male fate[49] (FIG. 1d). Commitment to male fate is also reinforced through positive feedback loops that later include the production of testosterone by the steroidogenic lineage. This mutually antagonistic system works to canalize development along one pathway while shutting down the other. In theory, any element that creates an imbalance between male and female networks could drive the system.

Birds depend on a ZW system with variations

Birds also use a stable pair of sex chromosomes for determining sex. However, unlike mammals, birds employ a ZZ/ZW chromosomal system, in which the female is the heterogametic sex. These sex chromosomes evolved from a completely different set of autosomes than the XY chromosomes in mammals[50]. In birds, sex determination is controlled by the dosage of a gene on the Z chromosome known as doublesex and mab-3 related transcription factor 1 (*DMRT1*): males have two copies of *DMRT1*, whereas females have only one. Introduction of viruses overexpressing *DMRT1* in the ZW (female) chicken gonad can drive male development, whereas suppression of *DMRT1* expression via viral transduction of a short hairpin RNA leads to sex reversal of ZZ (male) animals to female[51,52]. This is particularly interesting because *DMRT1* is a vertebrate orthologue of the doublesex and mab-3 (DM) gene family that is associated with sex differentiation in *D. melanogaster* and *Caenorhabditis elegans*[53]. Members of this family have evolved repeatedly to control sex differentiation in many species[54,55], although their role in mammals is to maintain the male pathway once it is initiated[56,57]. Despite the presence of a strong ZW genetic system in chickens, ZZ male eggs can be sex-reversed to female by the application of oestrogen during the critical period of gonad formation and commitment to testis or ovarian fate. Sensitivity to oestrogen is a characteristic of most egg-laying species.

Birds can develop as gynandromorphs in which the plumage, genitalia and other sexual dimorphisms are divided bilaterally into male characteristics on one side and female characteristics on the other. Although gynandromorphs have been reported in many species, they are most common in birds and arthropods (including butterflies and lobsters)[55,58]. Many possible mechanisms have been proposed to explain the origin of gynandromorphs; however, double fertilization of a binucleate egg may be the most common[59,60].

Investigations of a group of gynandromorphic chickens revealed that they were ZZ/ZW chimaeras, in which the male side of the chickens had a high proportion of ZZ cells, and the female side had a high proportion of ZW cells[61]. Based on the Jost paradigm, circulating sex hormones would be expected to pattern differentiation as uniformly male or female, regardless of the genotype of the cells. However, the gynandromorph results suggest that the chromosomal constitution of cells in birds influences their perception of the hormone environment — in other words, as in *D. melanogaster* and *C. elegans*, individual cells across the animal know their sex by their sex chromosome constitution[62]. This is not the case in eutherian mammals, where XX individuals can be fully sex-reversed to a phenotypic male if the gonad is induced to differentiate as a testis[10], indicating that the sex chromosome constitution of individual cells of mammals does not have a cell-autonomous influence over gonadal sex determination or differentiation of the sex ducts and genitalia.

Metatherian mammals are intermediate in this respect. Gonadal sex determination depends on *Sry*[63], and most secondary sex characteristics depend on the fate of the gonad[64,65]. However, in the tammar wallaby, the XX/XY sex chromosome identity of the cells in the common primordia for the pouch and scrotum determines which organ develops before primary sex determination in the gonad[66]. The tammar shares another characteristic with birds: whereas application of oestrogen does not affect primary sex determination in eutherian mammals, it does influence tammar gonad development if it is delivered to animals that are born a day earlier than normal, while the gonad is still plastic[67]. The lack of response to

Canalize
To channel development along a narrow path.

Heterogametic sex
The sex that produces two genetically different gametes.

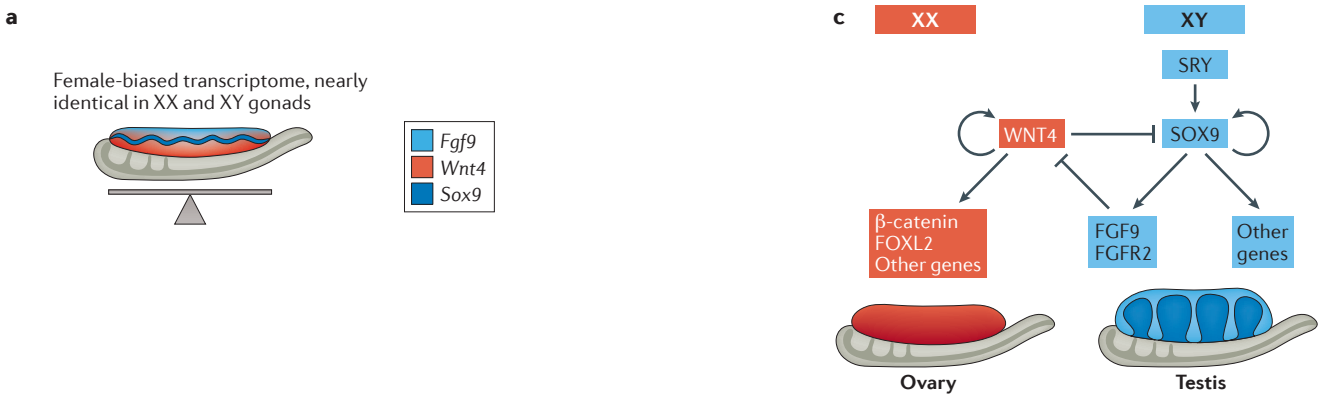
Gynandromorphs
Animals with both male and female phenotypic characteristics, often distributed bilaterally. Gynandromorphs occur in many species but are more common in arthropods and birds.

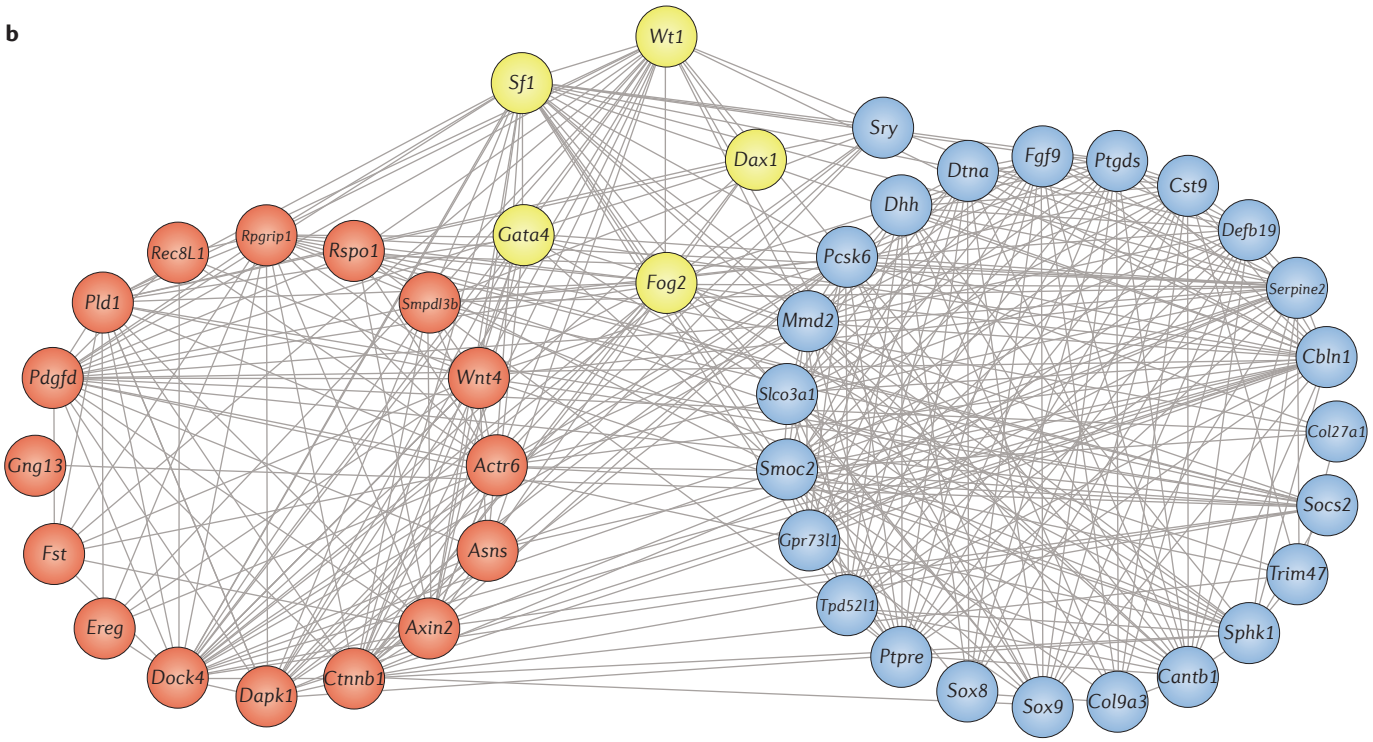
Chimaeras
Animals composed of two or more genetically different cell types (often arising from fusion of two fertilized eggs).

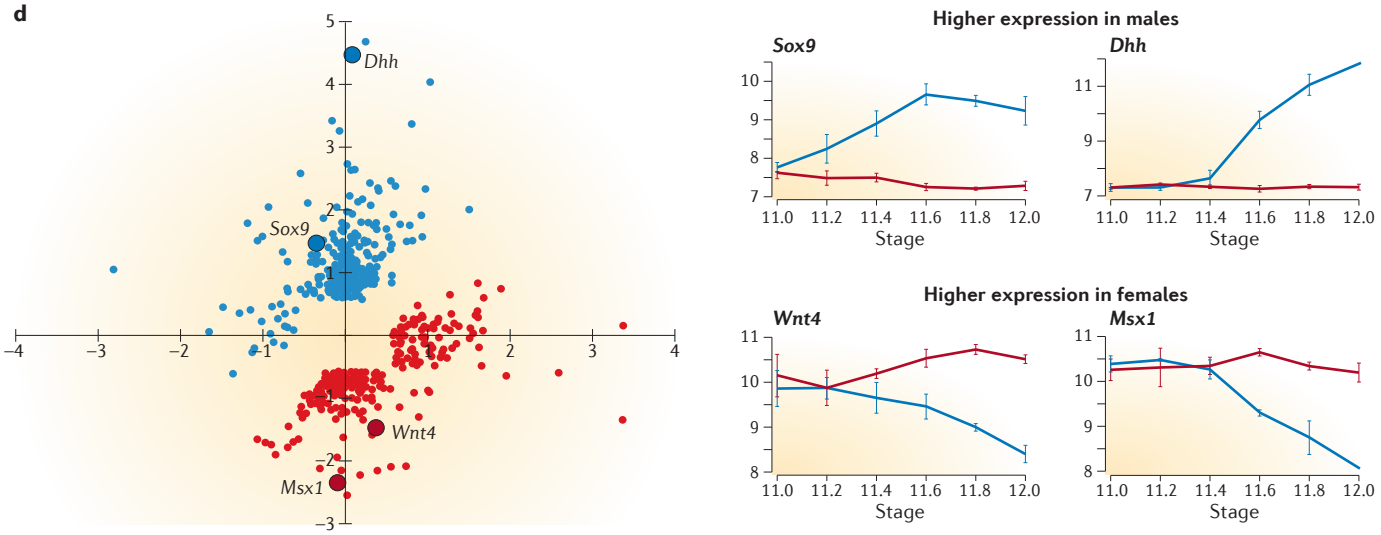
Eutherian mammals
Placental mammals that complete fetal development within the uterus.

Metatherian mammals
(Also known as marsupials). Placental mammals, such as kangaroos, that are born in mid-gestation and complete fetal development after birth.

Secondary sex characteristics
Characteristics that usually follow primary sex determination of the testis or ovary, for example, colouration, musculature, genitalia and sex ducts.







◀ Figure 1 | **Opposing signals control the fate of the mouse gonad. a** | At the bipotential stage (embryonic day (E)10.5–11.5), the transcriptional profiles of XX and XY gonads are nearly identical but show a bias towards genes associated with the female pathway. **b** | In E11.5 XY gonads, genes later associated with both the male (blue) and female (red) pathways are simultaneously expressed in two opposing sub-networks. Yellow circles indicate genes associated with both male and female pathways[145]. **c** | The primary role of the factor encoded by sex-determining region Y (SRY) is to activate SOX9. Between E11.5 and E12.5, SOX9 activates many targets in the male pathway, including fibroblast growth factor 9 (FGF9) and fibroblast growth factor receptor 2 (FGFR2), whose primary role is to block *Wnt4*, which would otherwise block SOX9 and activate the female pathway through β-catenin, forkhead box L2 (FOXL2) and other female factors. **d** | Plot reporting the change in expression of sexually dimorphic genes in the male (blue) and female (red) pathways in the XY gonad (Y-axis) and in the XX gonad (X-axis) between E11.0 and E12.0. In the XY gonad, male pathway genes are actively upregulated while female pathway genes are actively downregulated. Approximately half of the genes associated with the female pathway become sexually dimorphic through active downregulation in the XY gonad rather than through upregulation in the XX gonad. Part **d** is adapted from REF. 167.

oestrogen in eutherian mammals suggests that embryos that complete gonadal development within a uterine environment have evolved a mechanism to resist the influence of maternal oestrogen; otherwise, males would be very hard to come by.

Ectotherms transition between GSD and ESD

In contrast to the relatively stable GSD systems in endotherms, such as birds and mammals, sex determination systems in ectotherms exhibit a continuum of genetic and environmental mechanisms, including temperature-dependent sex determination (TSD) and susceptibility to hormone influence. Within any phylogenetic class, examples of XX/XY, ZZ/ZW, TSD and hormone regulation exist[9] (FIG. 2a). Frequent evolutionary transitions between GSD and TSD have been observed in the phylogenetic trees for turtles and lizards using classic cytological techniques to identify sex chromosomes[7]. Restriction site-associated DNA sequencing (RAD-seq) was used to identify cryptic sex chromosomes and to define approximately 25 transitions between XX/XY, ZZ/ZW and TSD systems among 12 gecko species[68] (FIG. 2b), suggesting a very low transition barrier.

TSD has been studied most extensively in reptiles. TSD is a class of ESD in which the incubation of the egg at different temperatures during the window of development when the gonad forms biases the percentage of male or female offspring[69]. The temperature that produces >90% males usually varies from the temperature that produces >90% females by only ~5 °C and is different among species. In some species, the higher temperature leads to females (for example, the red-eared slider turtle, *Trachemys scripta*); in others, the higher temperature leads to males (for example, the American alligator, *Alligator mississippiensis*); and in still others, the temperature extremes produce almost all females, while intermediate temperatures produce varying ratios of males (for example, the leopard gecko, *Eublepharis macularius*). All these patterns are likely to be part of the same U-shaped curve constricted by viability limits[70].

It seems unlikely that sex in any species is determined purely by TSD. In species where no chromosomal or genetic differences have been identified between sexes, such as *T. scripta*, a pivotal temperature (PvT) is defined as the temperature at which 50% of the eggs hatch as male and 50% hatch as female. In *T. scripta*, if the undifferentiated gonads are removed from an embryo and each gonad is cultured independently at the PvT, pairs of gonads show a strong tendency to follow the same pathway, suggesting that sex determination is not stochastic in the absence of thermal control. This tendency implies the existence of an underlying system (perhaps involving genetic or maternal influences) that drives sex determination in the absence of thermal extremes[71].

A molecular explanation for how temperature impacts the sex determination pathway is not known for any species, but theoretically, it must influence whether the male pathway surpasses the threshold for maleness set by the opposing female pathway[72,73]. This could be a protein variant that is part of the male or female pathway (for example, a transcription factor, enzyme, ion channel or epigenetic regulator) whose activity is temperature dependent, so that at one end of the range it induces maleness, but at the other end of the range, its activity is too low to antagonize the female pathway (two examples are diagrammed in FIG. 2c,d).

In theory, transitions between sex-determining mechanisms should be more likely in species with poorly differentiated sex chromosomes, which may explain the very rapid phylogenetic transitions in some reptiles. The presence of heteromorphic sex chromosomes should limit these transitions owing to the accumulation of genes that favour sexual fitness, as well as other mechanisms that regulate meiosis and dosage compensation in the heterogametic sex[74].

However, even reptile species with well-differentiated ZZ/ZW or XX/XY sex chromosomes can be sex-reversed by temperature during embryonic development[8,75]. Investigation of *Pogona vitticeps*, a species of bearded dragons, captured the transition of a lizard with a ZZ/ZW system to a TSD species in the wild. In this case, ZZ males incubated at high temperatures in the wild were sex-reversed to fertile females that were able to breed with ZZ males. This rapid transition due to climate conditions eliminated the W chromosome from this group of dragons in one generation[8,73]. A second case was documented in the Eastern three-lined skink, *Bassiana duperreyi*, which has a well-differentiated XX/XY GSD system. In this case, XX animals living in cold conditions were sex-reversed to male[75]. In both examples, the homogametic sex was sex-reversed; the lack of apparent sex reversal in the heterogametic sex prevents mating between individuals of the same heterogametic genotype and hence eliminates the disadvantage of producing nonviable WW or YY offspring[73].

The ability of temperature to dominate sex determination is a serious concern as the mean temperatures rise across the globe. Species with ESD are more likely to experience variable sex ratios from season to season (which may in some instances be an advantage). Importantly, deviations from the optimum sex ratio favour the invasion of a novel allele that produces individuals of the under-represented sex, shifting the sex ratio back towards the optimum[76]. The interaction between

Temperature-dependent sex determination
(TSD). One class of environmental sex determination in which sex determination is driven by temperature effects during a window of development.

Restriction site-associated DNA sequencing
(RAD-seq). Random sequencing of genomes anchored at restriction sites. The method is designed to screen the genome to uncover variations that show a high association with specific groups (for example, phenotypic males or females).

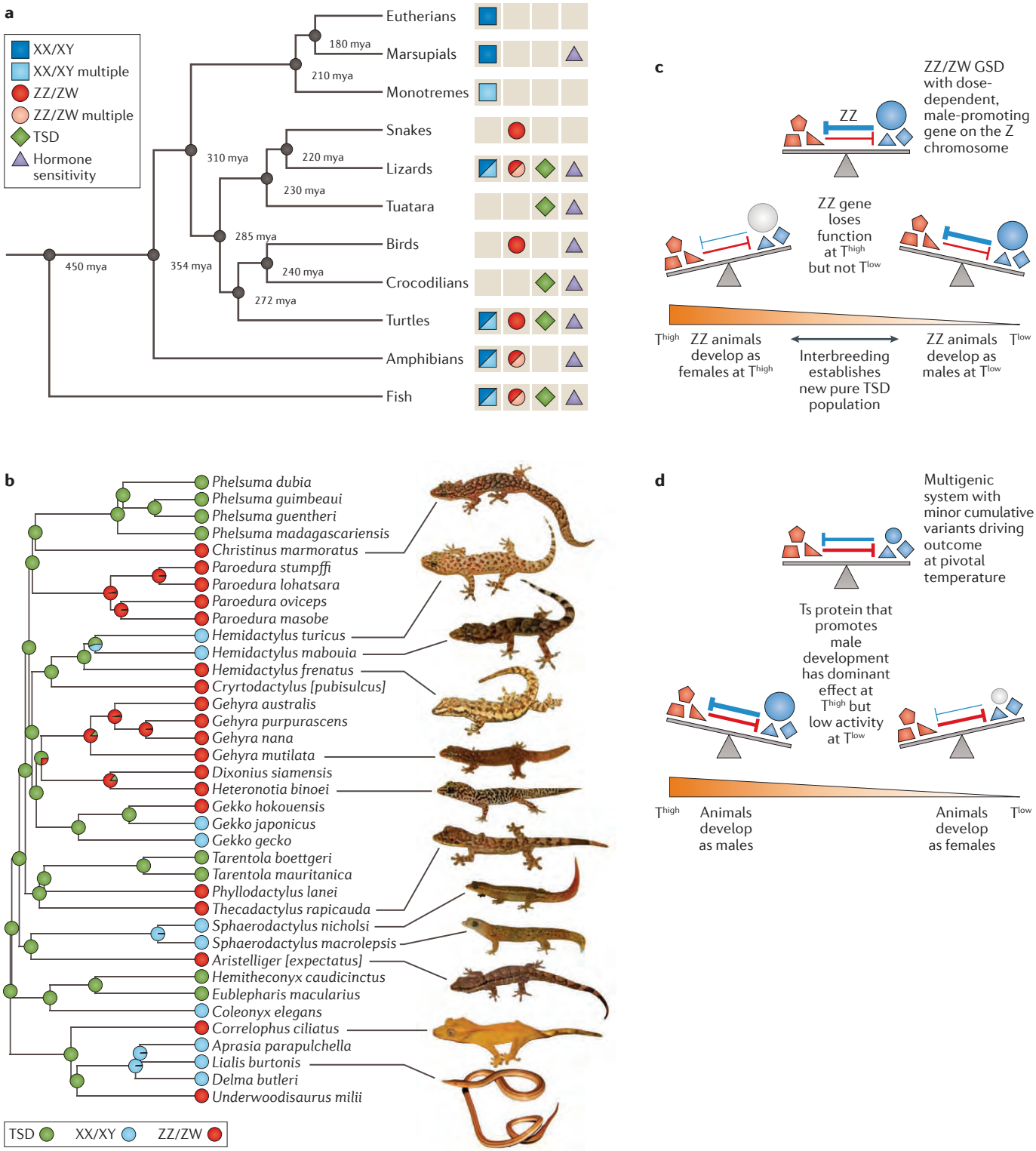


Figure 2 | **Transitions between sex-determining mechanisms are common.** **a** | More than one mechanism (genetic sex determination (GSD), environmental sex determination (ESD) and hormone sensitivity) can operate in a single class, often within individuals. **b** | Frequent transitions between sex-determining mechanisms. XX/XY and ZZ/ZW GSDs, as well as temperature-dependent sex determination (TSD), were detected among 12 gecko species using restriction site-associated DNA sequencing (RAD-seq). **c**,**d** | At the bipotential stage, male and female pathways are competing in the gonad. Example of the superimposition of TSD on an existing ZW/ZZ system in which a Z-linked gene whose dosage promotes the male pathway loses function at high temperature (T^{high}) so that ZZ animals develop as females at T^{high} but as males at low temperature (T^{low}) (part **c**). Example of the superimposition of TSD on an existing multigenic system, where the cumulative effects of multiple minor variants result in an ~50:50 sex ratio at a pivotal temperature (PvT) (part **d**). If a temperature-sensitive (Ts) protein associated with the male pathway arises, it can override the female pathway at one temperature, for example, T^{high}, but not at T^{low}, where development may be female-biased. Part **a** is adapted from REF. 9. Part **b** is adapted from REF. 68. mya, million years ago.

Dimorphic expression
Expressed differently between the two sexes.

GSD and ESD may explain the rapid turnover in sex-determining mechanisms and may be an important driver of the evolution of sex chromosomes, speciation events, adaptability and viability during climate change[73].

Rapid evolution of master regulators in fish

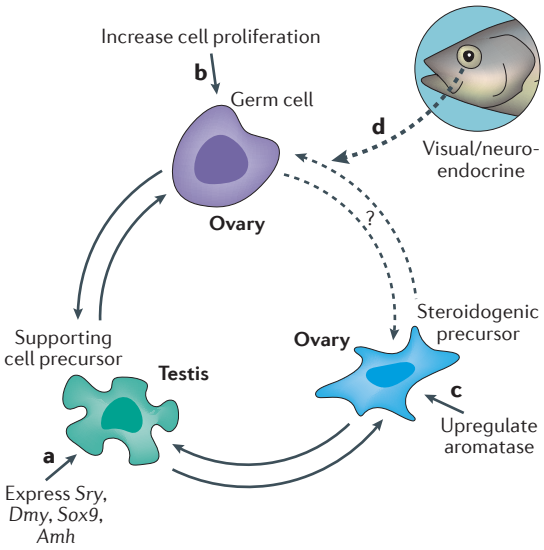
Fish also exhibit a continuum of GSD and ESD mechanisms, with sex-modifying environmental factors including temperature, population density and visual cues. Some fish, including medaka, rainbow trout and pejerrey, employ an XX/XY male heterogametic system, which evolved independently of the XX/XY system in mammals. Others, such as the Chinese tongue sole, use a ZZ/ZW system, and many closely related species, including tilapia[77], ricefish[78] and stickleback[79], show both types of heterogamety[80]. Even within the XX/XY systems, the gene that initiates male development varies. The *Dmy* (also known as *Dmrt1y* and *Dmrt1by*) gene is involved in somatic sex determination in two XX/XY species of the *Oryzias* genus medaka, *Oryzias latipes* and *Oryzias curvinotus*[81,82], where it evolved from *Dmrt1* through duplication and translocation to the proto-Y chromosome. *Sox3*, another familiar player, is the master regulator in the Indian ricefish *Oryzias dancena*[83]. The recurrent evolution of several genes, including Dmrt- and Sox-family genes, as the master regulators of sex determination led to the proposal that a limited group of genes can play this role[84,85].

However, in the salmonid family, sexually dimorphic on the Y chromosome (*sdY*), a gene with similarity to interferon regulatory factor 9 (*irf9*)[86], which has no previous association with sex determination, has evolved at the top of the cascade, suggesting that there may be more variation at the top of the cascade than previously appreciated[87]. In *Oryzias luzonensis*, a species of medaka closely related to those that use *Dmy*, gonadal soma-derived growth factor (*gsdf*) acts as a dosage-dependent master regulator of sex determination. Whereas both Dmrt-related genes and Sox-related genes encode transcription factors that act in the somatic cells of the early gonad to initiate dimorphic expression, *gsdf* encodes a member of the transforming growth factor-β (TGFβ) superfamily and acts as a diffusible signal to control the timing and rate of proliferation of germ cells[88], which controls sex determination in many fish (BOX 2). The *Amh* and *Amhr2* genes, which encode another TGFβ factor and its receptor, have also been implicated as sex-determining genes. An orthologue of *Amh, amhY*, has been identified on the Y chromosome of the Patagonian pejerrey *Odontesthes hatcheri*[89], whereas *amhr2* acts as

Box 2 | Sex determination can initiate in any cell type in the gonad

In mammalian systems, primary sex determination occurs in the supporting cell lineage. Sex-determining region Y (*Sry*) activates pathways that initiate Sertoli cell differentiation and repress the alternative granulosa cell fate (see the figure, part **a**). Once the fate of the supporting cells is established, they drive the differentiation of all other cell lineages in the gonad. The fate of germ cells is regulated by and matched to the somatic environment in which they develop[154,155].

Current data in zebrafish and medaka fish indicate that germ cell number can drive sex determination in these species (see the figure, part **b**). In zebrafish, if germ cells are depleted during the first day of development, all fish develop as sterile males[156–159]. If germ cells are depleted later, as occurs in the Fanconi anaemia mutation, *fancl*, adult females sex-reverse to phenotypic males and can become fertile if some of the germline stem cells persist and populate the testis[99]. Although zebrafish is not a typical environmental sex determination species, the sensitivity of sex determination to germ cell number may explain the impact of harsh environmental conditions, such as stress or poor nutrition, which favour males, perhaps by reducing germ cell populations[97]. In the medaka *Oryzias latipes*, which has an XX/XY genetic sex determination system, sex is strongly influenced by the number of germ cells that arise during embryonic development. When germ cells are depleted, fish develop with a male phenotype; by contrast, when the number of germ cells is amplified, for example, in the *hotei* mutant, fish develop as females[160], regardless of their sex chromosome constitution. These studies indicate that germ cells, and perhaps specifically oocytes, produce a signal (or signals) that acts upon the somatic gonad to promote the maintenance of a female developmental fate[161,162].



In species that are sensitive to oestrogen, upregulation of aromatase, presumably in the steroidogenic lineage, can drive sex determination (see the figure, part **c**), whereas in sex-reversing fish, the visual and neuroendocrine systems drive sex determination (see the figure, part **d**), although the primary affected cell type in the gonad is not yet determined.

In XY mice that have been sex-reversed to female (that is, by loss of *Sry*), XY germ cells can produce oocytes in an ovary (albeit with a low efficiency). In fact, the Y chromosome can be transmitted in a haploid oocyte[163,164]. However, XX germ cells that find themselves in a testis are blocked in male meiosis by a checkpoint requirement for pairing between the X and Y chromosomes[165]. The ability to switch from making oocytes to making sperm may be characteristic of species with poorly differentiated sex chromosomes, in which there are no pairing or dosage compensation imbalances between the sexes, and accumulation of few genes that favour fertility of one sex or the other. The figure is adapted from REF. 166.

a sex-determining gene in several species of pufferfish of the *Takifugu* genus[90]. Growth differentiation factor 6 (*gdf6*), encoding another TGFβ factor, acts as the Y-linked sex determination gene of killifish[91]. AMH is well known in mammals for its role in the regulation of Müllerian duct regression[92], but loss-of-function mutations do not affect gonadal sex determination, suggesting that this role was lost in the mammalian lineage[93].

Many fish experimentally determined to be XX/XY or ZZ/ZW have homomorphic sex chromosomes that are not well differentiated but nonetheless carry a gene that controls sex determination[94]. In other fish, sex chromosomes have not been identified, as in the present laboratory strains of zebrafish (*Danio rerio*). Multiple genes that influence sexual fate have been identified in zebrafish, suggesting that domesticated strains use a multigenic sex determination system but lack a single strong genetic determinant[95,96]. Surprisingly, investigation of wild strains revealed a robust ZZ/ZW system with a strong sex-linked single nucleotide polymorphism (SNP) near the telomere of chromosome 4, likely to be the wild sex chromosome[97]. It appears that domesticated strains lost the wild sex determination system, which uncovered alternative mechanisms to control sex, similar to what occurred in *P. vitticeps*[8]. These examples illustrate the remarkable evolvability of the system when faced with the compelling problem of generating two sexes to perpetuate the species.

Switching sex in adult life

Fish have a fluid sexual identity. The common laboratory model, zebrafish, is a gonochoristic species, with distinct adult males and females that do not normally sex-reverse in adult life. However, they are classified as transient hermaphrodites because all larval fish initially produce oocytes before differentiation to a functional male or female[98,99].

Many fish, including the bluehead wrasse (*Thalassoma bifasciatum*)[100], Potter's angelfish (*Centropyge potteri*)[101] and the lyretail anthias (*Anthias squamipinnis*)[102], show natural sequential hermaphroditism, where male and female phases alternate in adult life, depending on developmental stage, environment and social cues. Sequential hermaphroditism occurs in at least 27 families distributed across 9 teleost orders, suggesting that it has evolved repeatedly. Changes can be protogynous, protandrous or bidirectional. Within each social group, there are fertile females, one dominant male and immature males, whose maturation is suppressed by the behaviour of the dominant male (FIG. 3A,B). If the dominant male is removed or blocked from view, another fish will become the dominant male. Sometimes an immature male takes on this role, and sometimes a mature female will undergo sex reversal, typically depending on size and social rank.

Sex change is often regulated by the visual and neuroendocrine systems. Although levels of oestrogen and 11-ketotestosterone are strongly correlated with the direction of the sex change and probably mediate remodelling of the gonad[103,104], dominant behavioural changes can occur very rapidly — within minutes to hours — long before gonadal changes occur[94,103,105,106]. Behavioural changes are mediated by neuropeptides, which may include gonadotropin-releasing hormone, kisspeptin, isotocin and arginine vasotocin. In some cases, environmental stress, such as temperature and population and/or social dynamics, may trigger masculinization of the gonad via increased cortisol production from the inter-renal gland[107] (FIG. 3A). Within the gonad, transcriptome analysis indicates that during either protandrous or protogynous sex change, shutdown of the existing transcriptional network is necessary to release suppression of the opposing network[108] (FIG. 4a). Signalling within the gonad converges on regulation of oestrogen as in birds and reptiles, although the order of genes in the cascade is not conserved. So far, the various types of sex change, the species-specific variations and the methodological differences among studies have prevented the identification of broadly representative mechanisms, but this is likely to change as more examples are studied at the molecular level[103,104].

Although functional sex reversal is unknown in mammals, some examples of seasonal plasticity and genetically induced sex reversal attest to the underlying plasticity of the system. There is a curious example of naturally occurring seasonal hermaphroditism in the mammalian species *Talpa occidentalis*, a mole native to the Iberian Peninsula. Female moles show seasonal variation in their sex hormones and gonad structure, but they breed as only one sex (female). Females live in solitary burrows during the non-breeding season. The medullary compartment of the ovary expands and produces high levels of testosterone, which results in partial masculinization of external genitalia and aggressive behaviour[38,109]. When the breeding season returns, the medullary compartment of the ovary contracts, the cortical region expands and

Homomorphic sex chromosomes
Sex chromosomes that are not morphologically distinguishable but nonetheless influence sex determination.

Multigenic sex determination
Sex determination that depends on multiple alleles segregating in the population.

Transient hermaphrodites
Animals that initially produce gametes of one sex, followed by full differentiation as male or female. Zebrafish all hatch producing oocytes, followed by maturation as functional males or females.

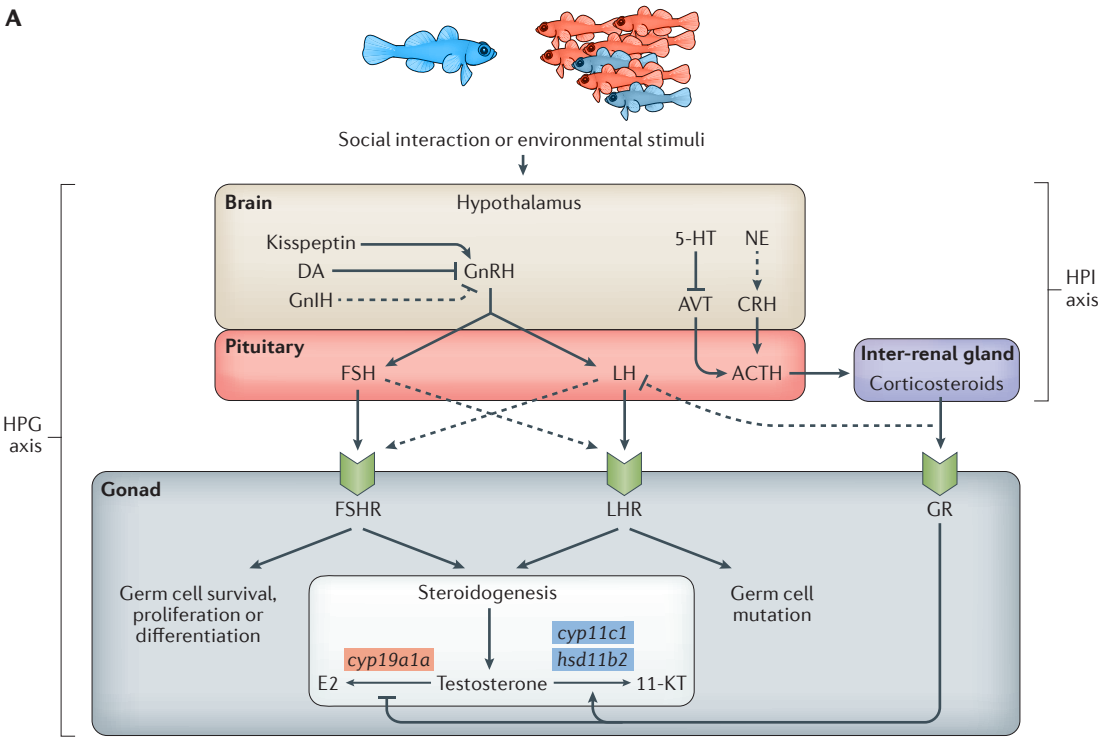
Sequential hermaphroditism
Functioning as one sex followed by a functional switch to the other sex.

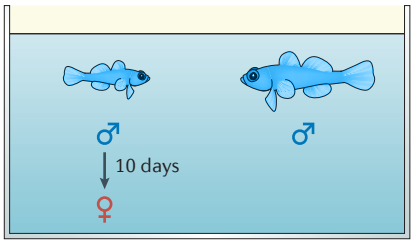
Protogynous
Among species that change sex as adults, those that are first female, then male.

Protandrous
Among species that change sex as adults, those that are first male, then female.

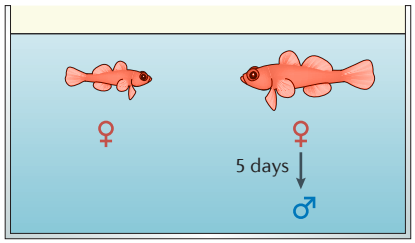
Inter-renal gland
In fish, the functional equivalent of the mammalian adrenal cortex, producing corticosteroids and regulating water metabolism and stress.

Figure 3 | **Many fish use visual cues for sex determination. A** | Social interactions and environmental stimuli operate through the hypothalamus–pituitary–inter-renal (HPI) axis via neuroendocrine and steroidogenic factors, including kisspeptin, dopamine (DA), gonadotropin-releasing hormone (GnRH) and arginine vasotocin (AVT). Follicle-stimulating hormone (FSH) and luteinising hormone (LH) may stimulate germ cell survival, proliferation or maturation, whereas corticosteroids produced by the adrenal gland act in the gonad to block the aromatase enzyme, encoded by *cyp19a1a*, which converts testosterone to 17β-oestradiol (E2) in the female developmental pathway, and promote the enzymes encoded by *cyp11c1* and *hsd11b2*, which drive the conversion of testosterone to 11-ketotestosterone (11-KT) production in the male developmental pathway. **B** | Sex change in fish is usually based on size and behaviour. **Ba**,**Bb**,**Bc** | The larger of two male fish remains male, while the smaller becomes a female whether fish are housed together in a single tank or separated by a glass enclosure. **Bd**,**Be**,**Bf** | In the absence of males, the largest female changes sex to male. Part **A** is adapted from REF. 168. Part **B** is adapted from REF. 169. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; GnIH, gonadotropin inhibitory hormone; FSHR, FSH receptor; LHR, LH receptor; MIH, maturation-inducing hormone; NE, noradrenaline; 5-HT, serotonin.

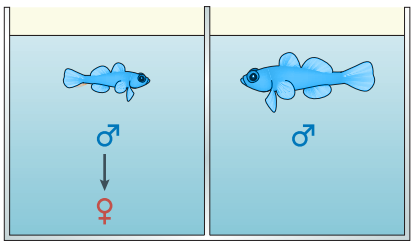




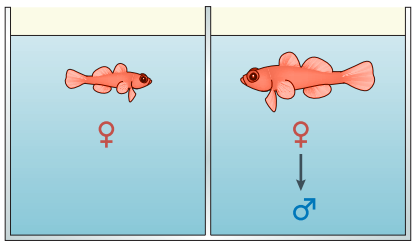
Ba
Condition: Two males of different size.
Result: Smaller male changes sex.



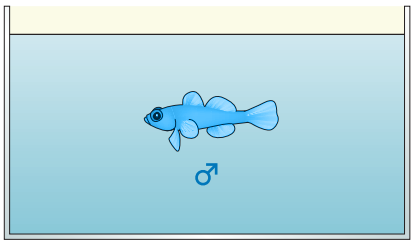
Bd
Condition: Two females of different size.
Result: Larger female changes sex.



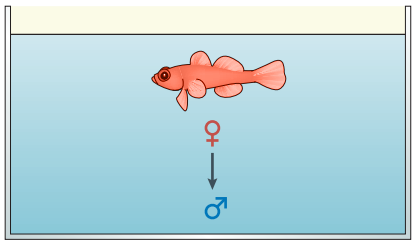
Bb
Condition: Two males of different size are kept in glass-separated aquarium. Fish can see each other, but not touch.
Result: Smaller male changes sex.



Be
Condition: Two different sized females are kept in glass-separated aquarium. Fish can see each other, but not touch.
Result: Larger female changes sex.



Bc
Condition: Single male isolated.
Result: No sex change.



Bf
Condition: Single female isolated.
Result: Female changes sex.

CTCF
(CCCTC-binding factor). A chromatin-binding factor that mediates repressive chromatin domains.

produces oocytes, the genitalia are feminized, and the females become receptive to males[110].

Adult mammals do not spontaneously undergo sex reversal. However, various genetic perturbations can destabilize the commitment to Sertoli and granulosa cell fate in adult life. For example, loss of *Dmrt1* in adult Sertoli cells leads to derepression of forkhead box L2 (*Foxl2*), a marker of granulosa cell fate[111]. Similarly, loss of *Foxl2* in granulosa cells leads to derepression of *Sox9* and at least partial transdifferentiation of the ovary to testis identity[112]. These findings suggest that cells not only 'remember' their alternative fate but also that active and ongoing repression of that alternative fate is necessary, even in adult life.

Epigenetic mechanisms

There is evidence for involvement of epigenetic mechanisms in both the initiation and in the stabilization and maintenance of sex determination in humans and mice. For example, an unmethylated CTCF-binding site was mapped upstream of the human *Sry* gene in white blood cells and was associated with enrichment of histone H3 lysine 9 trimethylation (H3K9me3) marks, consistent with recruitment of Polycomb repressive complex 2 (PRC2) to silence the locus[113]. Consistent with the idea that activation of *Sry* requires depletion of H3K9me3, XY mice deficient for the H3K9-demethylating enzyme JMJD1A show an increase in H3K9 dimethylation and a decrease in the activating mark of H3K4 trimethylation across the locus, leading to a high frequency of sex reversal[114]. Further evidence for epigenetic regulation of *Sry* came from studies of chromobox protein homologue 2 (*Cbx2*). Loss of function of *Cbx2* in mice led to hypoplastic gonads and male-to-female sex reversal, which could be rescued by forced expression of *Sry*[115,116]. A role for CBX2 as an activator of *Sry* was unexpected, given the classical role of the protein in PRC1[117], although the effect on *Sry* could be mediated through repression of a repressor.

Consistent with the role of *Cbx2* in mice, a patient with male-to-female sex reversal carried a mutation in *CBX2*[118]. Chromatin immunoprecipitation of CBX2 in Sertoli-like cells identified genomic targets associated

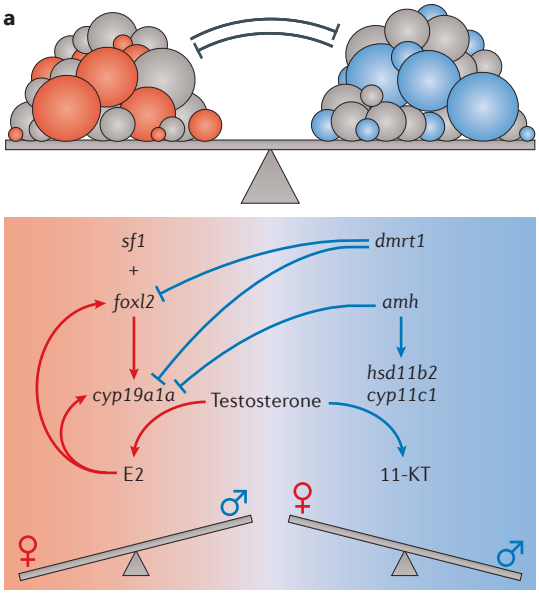


Figure 4 | **Many factors of different magnitudes may contribute to a 'parliamentary decision' in the gonad.** Antagonism between the male and female pathways as well as multiple feedback loops that reinforce the decision are a common feature in all sex-determining systems, but the specific genes involved in the primary decision vary. **a** | In fish, doublesex and mab-3 related transcription factor 1 (*dmrt1*) and anti-Müllerian hormone (*amh*) characterize the male pathway and repress the female pathway, and forkhead box L2 (*foxl2*) and *cyp19a1a* (encoding aromatase, which converts testosterone to the oestrogen 17β-oestradiol (E2)) characterize the female pathway and repress the male pathway. As in birds, many reptiles and crocodilia, the system in fish converges on whether or not oestrogen is produced from the testosterone precursor, which is often the readout for stable commitment to the female (red) or male (blue) pathway. In these species, adding or blocking oestrogen can serve as an entry point and override the molecular pathway. Expression of *hsd11b2* and *cyp11c1*, which encode two enzymes downstream in the male pathway, promotes the conversion of testosterone to the active androgen, 11-ketotestosterone (11-KT), which depletes its availability for conversion to E2. **b** | Many genes recur in different species; however, their dimorphic expression does not arise in the same linear order. Cassettes of genes in the male pathway shift in the order in which they become dimorphically expressed between the red-eared slider turtle and the mouse. Genes are ordered based on their earliest acquisition of differential expression in the gonadal time course in the turtle (left; stages 15–18) and mouse (right; embryonic day (E) 11.2–11.8). Each cassette is labelled with a different colour and connected across species with a diagonal box. Further investigations may help to untangle the transcriptional cassettes associated with the differentiation of each cell type and with the organogenesis of the testis and ovary. Female genes show similar shifts. Part **b** is adapted from REF. 130.

with both male and female pathways[119]. Several lines of evidence suggest that silencing the female pathway is required to establish the male pathway and vice-versa[49,120]. In cases where Wnt signalling is not silenced, the male pathway is not stabilized, despite the activation of *Sry* and *Sox9*[25,121]. CBX2 may be involved in blocking expression of genes associated with female fate, which would otherwise disrupt commitment to male fate.

Epigenetic regulation could mediate the influence of the environment on sex determination. Studies have linked environmental toxicants and dietary changes to epigenetic mechanisms. For example, sodium butyrate, a short-chain fatty acid produced by bacteria in the gut, acts as a histone deacetylase inhibitor that can affect the epigenetic status of genes in the European sea bass *Dicentrarchus labrax*[122]. Exposure to polychlorinated biphenyls (PCBs), which have been used as a biomarker of environmental contamination, led to epigenetic changes that were correlated with altered transcriptional profiles of genes responsible for gonadal differentiation. These changes biased sex ratios towards female in the red-eared slider turtle[123].

Changes in DNA methylation patterns have been correlated with exposure to male-producing temperatures (MPT) or female-producing temperatures (FPT). DNA methylation in the promoter of the aromatase gene (*cyp19a1a*), which seems to act as a convergent readout signifying commitment to the female pathway in both fish and reptiles, responds to temperature in the European sea bass, is inversely correlated with expression and represents a potential molecular link between the environment and sex determination[124]. Similarly, in the red-eared slider turtle[125] and the American alligator[126], a substantial increase in DNA methylation was detected at the aromatase gene promoter at MPT and was correlated with the absence of transcription. Shifting embryos from MPT to FPT resulted in demethylation of the sites and activation of expression. However, it has been difficult to determine whether methylation patterns represent a cause or effect of aromatase activation.

Another study in the half-smooth tongue sole *Cynoglossus semilaevis* suggests a causative effect of DNA methylation. Investigators compared the gonad-wide methylome in ZZ and ZW fish and identified differences at key sex-specific genes, such as *dmrt1*. When ZW females were sex-reversed to 'pseudomales' by temperature exposure, gonadal cells had male epigenetic marks. ZW offspring in the F1 generation (produced by a cross between a ZW pseudomale and a normal ZW female) retained male epigenetic marks in their gonadal cells, and ~90% spontaneously sex-reversed in the absence of thermal influence[127]. These results suggest that temperature resets heritable epigenetic marks and could override the female ZW genotype.

The aromatase promoter in female (ZW) chicken gonads is hypomethylated at the DNA level and characterized by a high ratio of H3K4me3/H3K27me3 residues relative to the same locus in male gonads, where the aromatase promoter is hypermethylated at the DNA level and characterized by a low ratio of H3K4me3/H3K27me3 marks. Curiously, however, these marks are only partially reprogrammed at the locus when ZZ-male chickens are sex-reversed to female by treatment with oestrogens, despite the fact that the animals are morphologically sex-reversed to female and express aromatase at female levels[128]. These findings partially dissociate aromatase expression from epigenetic programming of the locus, although it remains possible that patterns at only a few residues are critical.

Although investigation of the possible link between ESD and epigenetic regulation remains somewhat anecdotal, in both the American alligator[129] and the red-eared slider turtle[130], the histone demethylase *Kdm6b* was identified as an early responder to male incubation temperature and could provide a molecular foothold for a functional genetic investigation of the role of histone modifications in sex determination in ESD species.

Hierarchical pathways or emergent bistability

Early models of sex determination pathways attempted to account for the multiple chromosomal (XX/XY, ZZ/ZW) and non-chromosomal (for example, ESD) systems known to be involved by positing the existence of a 'master regulator' activating a hierarchical cascade of genes eventually leading to the differentiation of a testis or ovary from the bipotential primordium[131,132]. In these models, the sex determination cascade was predicted to have evolved from the bottom up. In other words, highly conserved transcription factor–binding site interactions govern the stable downstream cascade, but there is frequent evolution of new 'master genes' at the top that can initiate the pathway[133] (FIG. 5a).

Interestingly, conservation of an orderly downstream cascade has not proved to be the case. Although many of the same genes are expressed during gonadal sex determination in birds, mice, turtles and fish, there is no common hierarchy of expression in downstream pathways[134–136]. Instead, cassettes of genes show heterochronic shifts between species[130] (FIG. 4b).

Even in systems where a strong master regulator exists, genetic experiments indicate that the regulator can be replaced by a downstream gene. For example, both *Sox9* and *Dmrt1* can replace *Sry* as the master regulator of the male pathway in mammals[12,137,138]. The system can be manipulated further downstream by loss-of-function mutations in the male or female signalling pathways[23,24,139]. For example, males null for *Fgf9* undergo sex reversal to female. However, male development can be recovered if the female signalling molecule *Wnt4* is simultaneously deleted[25]. Loss of function of kinase genes can also lead to male-to-female sex reversal in mice and humans[140,141], whereas gain of function of female genes can override the male pathway[121,142]. These genetic experiments strongly suggest that there are multiple functional entry points into mammalian sex-determination pathways, as was demonstrated in classic experiments in *C. elegans*[143].

The network structure of the system may be highly permissive for rapid transitions in key regulators. Networks need not depend on a discrete upstream regulator but can canalize the pathway[144], no matter where in the network the signal initiates. It has been

Heterochronic
Expressed in a different sequence in development.

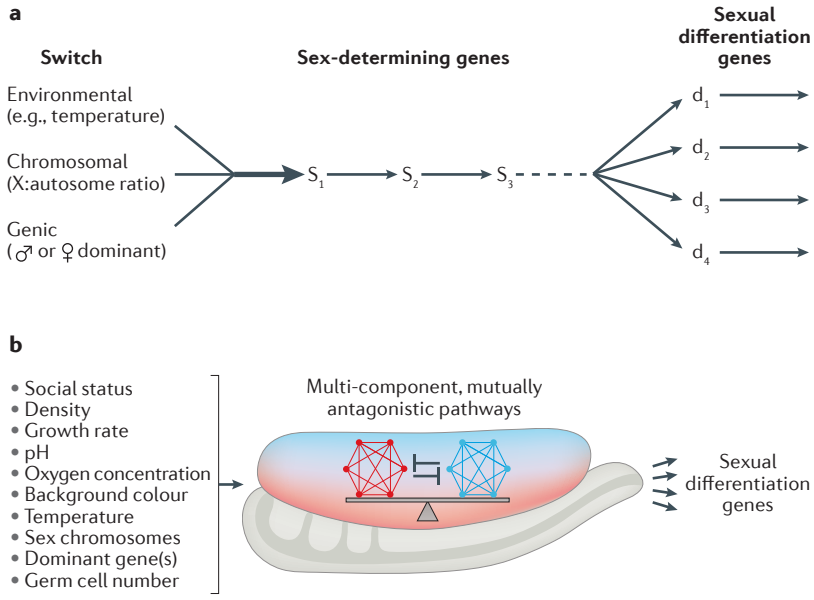


Figure 5 | **Old and new models for sex-determination mechanisms. a** | Sex determination in mammals was proposed to be a linear pathway built from the bottom up, with changes in the master regulator at the top of the cascade. **b** | An alternative model in which networks with multiple feedback loops replace linearity: antagonism between male and female networks is conserved, but components do not maintain linear order. Part **a** is adapted from REF. 132. Part **b** is adapted from REF. 136.

suggested that differentiation pathways and multilayered feedback loops work together in a non-hierarchical network to produce a male or female phenotype[136]. Although these networks can be strongly canalized by several major-effect loci, multigenic systems with contributions from multiple allelic variants may be common. In these systems, sex determination may be driven by a parliamentary decision resulting from multiple upstream and downstream elements that feed into a threshold decision process, driving a bistable outcome (FIG. 5b).

Mutual antagonism between the two possible outcomes is a critical feature of the network. Both genetic experiments and transcriptome analysis indicate that sex determination in mice is governed by antagonistic networks that promote male or female development and silence the alternative pathway[22,23,49]. Even in adult life, the fate of testis and ovarian cells is actively maintained by repression of the alternative fate[111,112]. Feedback loops occur at molecular, cellular and physiological levels and act to canalize male or female pathways. For example, molecular pathways within Sertoli cells and between other cells in the gonad act to stabilize expression of SOX9 (REF. 145). In the female pathway, germ cell commitment to meiosis favours ovarian development and stabilizes granulosa cell differentiation[146,147]. Although hormones do not regulate the early steps in mammalian sex determination, they later reinforce male versus female development. These findings are consistent with an interconnected network structure in which antagonism between networks and strong feedback regulation act to canalize the pathway once a threshold bias for one of the two fates exists.

Parliamentary decision
A decision resulting from the contribution of many factors.

Conclusions

The discovery of *Sry* in 1990 (REF. 11) triggered a revolution in our understanding of vertebrate sex determination as we began to define downstream pathways and gain a molecular foothold in the relatively well-studied systems in mammals, birds and a few reptiles and fish. More recently, advances in non-model organisms have been fuelled by genome and transcriptome analyses that make nearly any system genetically accessible. Schemes using CRISPR or viruses to perform loss- and gain-of-function experiments will soon provide functional answers in many organisms. Further investigations at the single-cell level may help to untangle the transcriptional cassettes associated with the differentiation of each cell type and those that control the organogenesis of the testis and ovary.

The bipotential nature of the gonad, the sex ducts and the genitalia creates plasticity with a special set of problems. One pathway must be established and coordinated across the entire organism, while the other is suppressed. Evolution has solved this problem in many ways. Mechanisms to determine sexual fate range from highly evolved sex chromosomes devoted to the task to the dominance of temperature and social cues in species where no strong genetic determinants have been identified. Even if a species has evolved a dominant genetic determinant, if that determinant is lost, a new mechanism emerges to resolve sex. Regardless of whether the system is triggered by a master regulator or by a parliamentary decision or whether the switch occurs in the brain or a somatic or germ cell lineage, a common theme is the existence of antagonistic signals that ensure canalization of one pathway or the other. This basic underlying principle may explain how plasticity is tolerated.

What is the evolutionary advantage of a plastic system that is not hard-wired? No one knows the answer to this question, but the survival of most species depends on the generation of males and females, which suggests that there must be an explanation. One possibility is that the system evolved to be permissive of sex reversal, which is a strong adaptive advantage when one sex is in short supply. Another possibility is that the plasticity in the system produces wide phenotypic variance within male and female categories, which may be adaptive in changing environments as long as phenotypic variance is counterbalanced by strong canalization to generate individuals who breed as one sex or the other.

The discovery that mammalian testis or ovary fate requires repression of the alternative state, even in adult life[111,112], was very surprising. A comparison of the epigenetic state of gonadal progenitors, early differentiating cells and adult cells may provide a chromatin-level view of the molecular nature of plasticity and its resolution. These studies may reveal how signalling and feedback loops coincide with changes in epigenetic states and transcriptional outcomes to drive sex determination and, by extension, yield insights into how many other bipotential progenitors manage this problem during development.

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Competing interests statement
The author declares no competing interests.

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"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	**Week 10**	**Spring Break**
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review

Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Sex Determination
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesdays & Thursdays
January 23, 2024
Week 3

Sex Determination

- History
- Jost model of sexual differentiation
  - Chromosomal sex
  - Gonadal sex
  - Phenotypic sex
- Gonadal development systems
  - Cell biology
  - Required genes
- How does chromosomal sex dictate gonadal sex?
  - Molecular cloning of testis-determining factor(s) (e.g. SRY)
  - Interactions of SRY and SOX genes
  - X chromosome sex determining factor DSS/DAX
  - Interactions SRY, SOX, DAX, SF1, and DMRT
- How does gonadal sex dictate phenotypic sex?
  - Müllerian Inhibitory Substance (MIS)
  - Androgen induced male differentiation
- Abnormal sexual differentiation
  - New potential sex determination genes
- Mechanisms of sex determination in other species

Required Reading

Wilhelm and Pask (2018) Genetic Mechanisms of Sex Determination, in: Encyclopedia of Reproduction 2nd Ed. Vol 3, Pages 245-249.

Capel (2017) Nature Reviews Genetics 18:675.

Spring 2024 – Systems Biology of Reproduction
Discussion Outline (Sex Determination)
Michael K. Skinner – Biol 475/575
January 25, 2024
Week 3

Sex Determination

Primary Papers:

1. Yamauchi, et al. (2014) Science 343:69-72
2. Bhandari, et al. (2012) PLoS ONE 7:e43380
3. Okashita, et al. (2019) Scientific Reports 9:13462
4. Tsuji-Hosokawa et al. (2022) Endocrinology 1;163(1):bqab217

Discussion

Student 4: Reference #1 above
- What are the genes on the Y required?
- What was the experimental design and methods?
- What conclusions are made on the future fate of the Y?

Student 5: Reference #2 above
- What are the downstream targets of SRY?
- What was the method used to identify the targets?
- Is SOX9 the only target of SRY that is important?

Student 6: Reference #3 above
- What is Tet2 and function in DNA methylation?
- What role does DNA methylation and histone modification have in sex determination?

Reference #4 above
- What was the experimental design?
- What observations were made on sex determination and SRY?

History and Jost

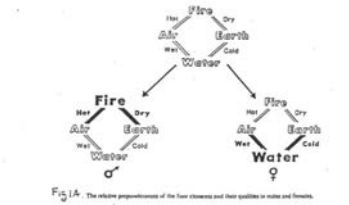


Fig 1A. The relative preponderances of the four elements and their qualities in males and females.

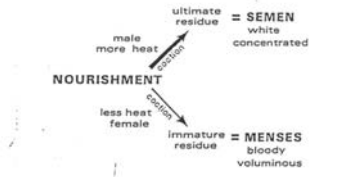
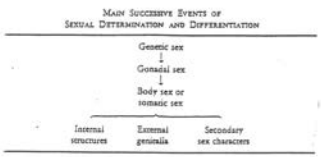


Fig 1B. The greater innate heat of the male allows for a greater degree of coction of the nourishment and hence a more concentrated white semen is formed. In the "colder" female coction is much less, leaving a more voluminous, less mature residue from which the menstrual fluid is derived.



Jost Model -

Alfred Jost, University of Paris

1940's & 1950's

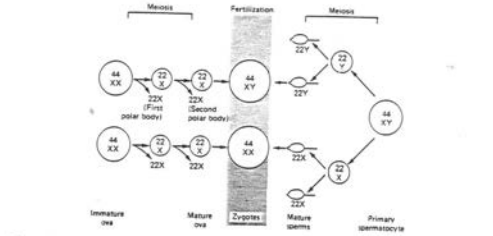
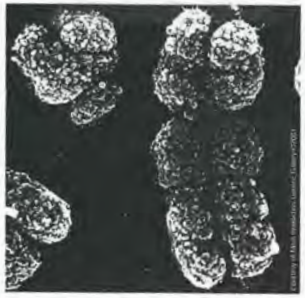
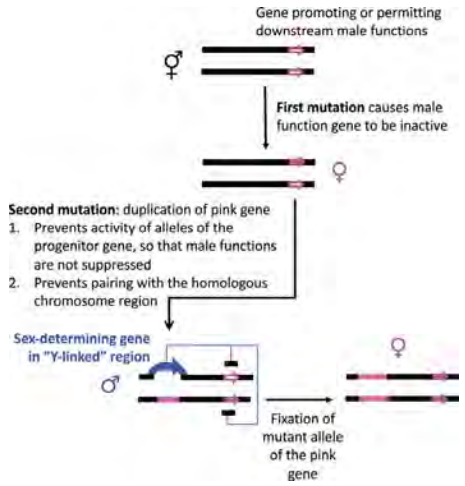


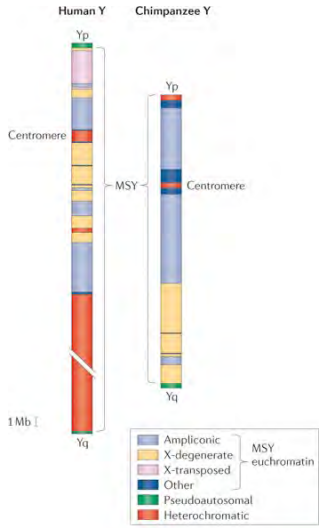
Figure 23–1. Basis of genetic sex determination. In the case of the 2-stage meiotic division in the female, only one body survives as the mature ovum. In the case of the male, the meiotic division results in the formation of 4 sperms, 2 containing the X and 2 the Y chromosome. Fertilization thus produces a 44XY (male) zygote or a 44XX (female) zygote.



Neutrality? The default starting point for human embryos may be a neutral one, not male or female as previously thought.

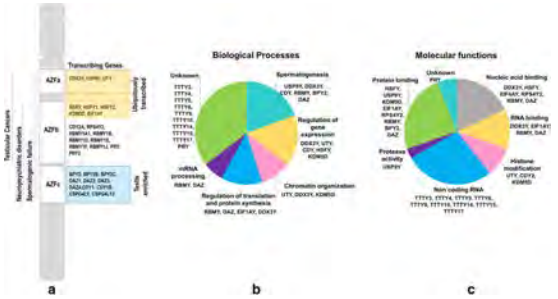
When and how do sex-linked regions become sex chromosomes?
Charlesworth D.
Evolution. 2021 Mar;75(3):569-581.



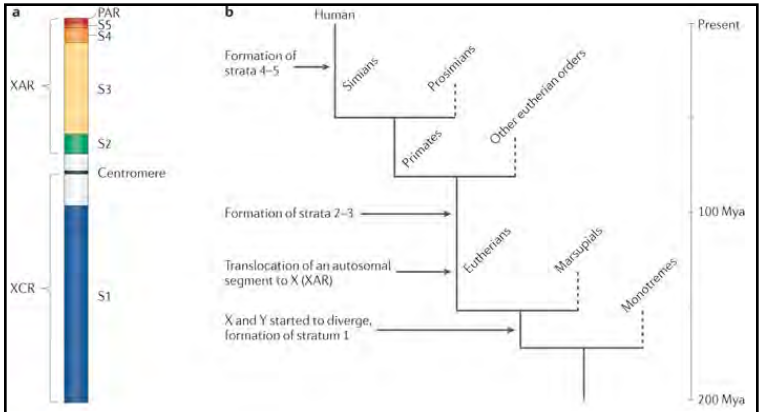


The human and chimpanzee Y chromosomes differ considerably in organization and structure despite the recent divergence of the two lineages (about 6 million years ago). Ampliconic regions contain massive palindromic arrays of multi-copy testis-specific genes. The X-degenerate regions denote segments homologous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

Consequences of Y chromosome microdeletions beyond male infertility.
Colaco S, Modi D.
J Assist Reprod Genet. 2019 Jul;36(7):1329-1337.



Transcribing genes within the AZF loci of the human Y chromosome, their biological processes and molecular functions. A. Transcribing genes in the AZF loci of the human Y chromosome where each block represents the three AZF clusters. The genes in the yellow overlaid block are ubiquitously transcribed while those in the blue block are generally testis-enriched. The associated phenotypic manifestations due to loss or gain of these gene/gene families is depicted by the lilac bars to the extreme left of the image. Tissue expression of the genes has been labelled adjacent to the boxes. B. Biological processes and C. Molecular functions of the transcribing genes within the AZF loci as predicted by Uniprot (https://www.uniprot.org, accessed on 15 Nov 2018). t **a.** T is depicted. lilac extreme **b.c**.

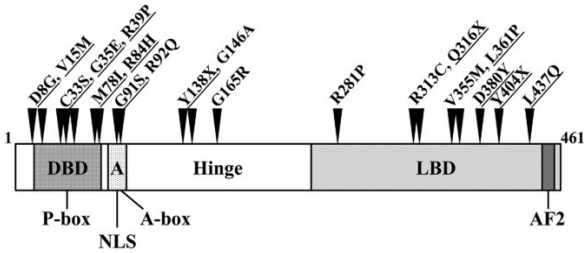


a | A schematic illustration of the human X chromosome. The evolutionary history of this chromosome includes a region defined as the X-conserved region (XCR) that is present on the X chromosome of marsupials but is autosomal in monotremes. This indicates that in therians (placental mammals plus marsupials)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

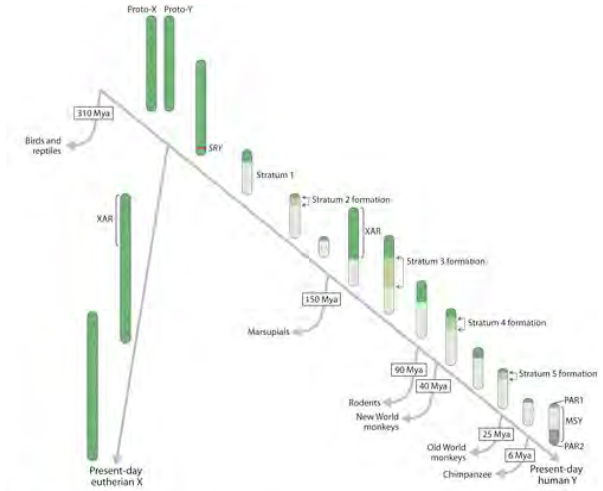
Mutant NR5A1/SF-1 in patients with disorders of sex development shows defective activation of the SOX9 TESCO enhancer.

Sreenivasan R, Ludbrook L, Fisher B, Declosmenil F, Knower KC, Croft B, Bird AD, Ryan J, Bashamboo A, Sinclair AH, Koopman P, McElreavey K, Poulat F, Harley VR.

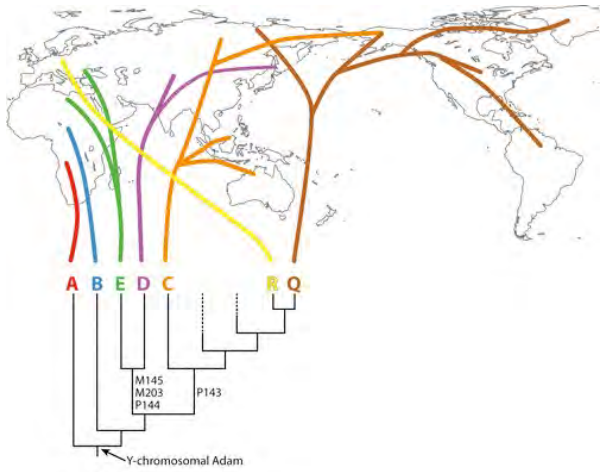
Hum Mutat. 2018 Dec;39(12):1861-1874.



Structure of wild-type SF-1 protein showing location of 20 mutants found in 46,XY DSD patients. Each amino acid substitution is coded according to severity of developmental phenotype (black for developmentally mild phenotypes with under-masculinized male genitalia; underlined for severe phenotypes with ambiguous or female genitalia). Numbers represent the first and last amino acid of the SF-1 protein. Functional domains of SF-1 are labeled as follows: DBD, DNA binding domain; P-box, proximal box; NLS, nuclear localization signal; A-box, accessory box; Hinge, hinge domain; LBD, ligand binding domain; AF2, activation function 2



Hughes JF, Rozen S. 2012.
Annu. Rev. Genomics Hum. Genet. 13:83–108



Hughes JF, Rozen S. 2012.
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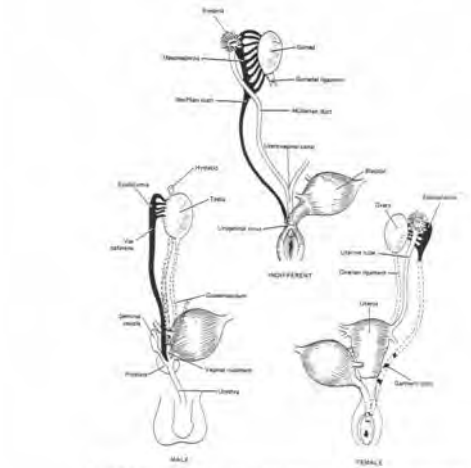
Gonadal Sex Determination



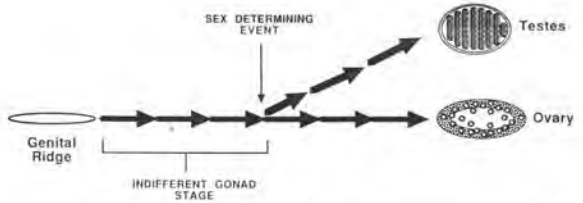
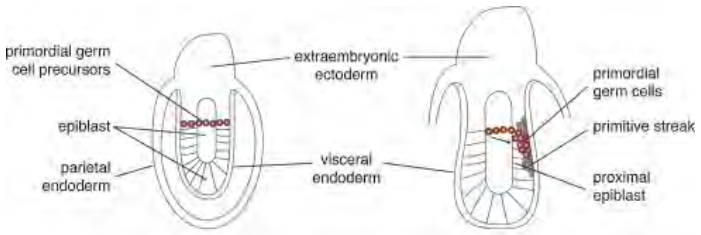
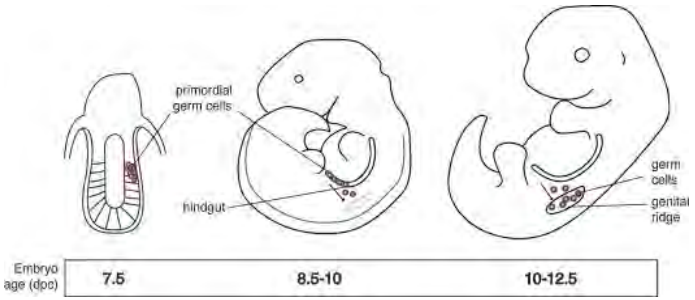
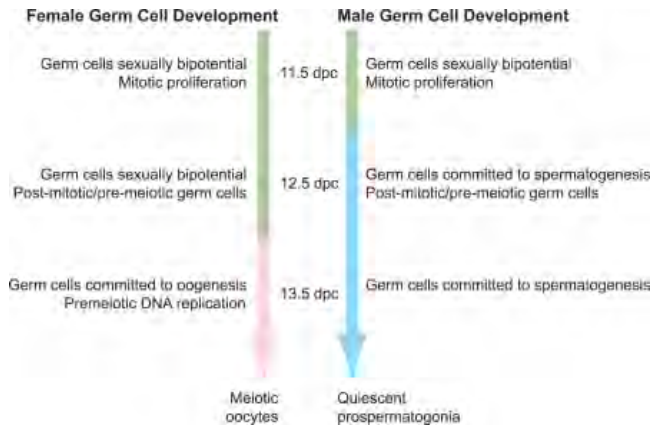
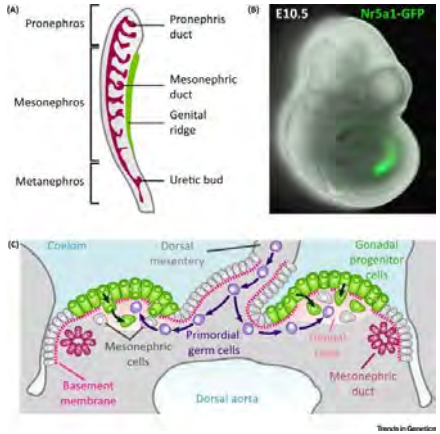


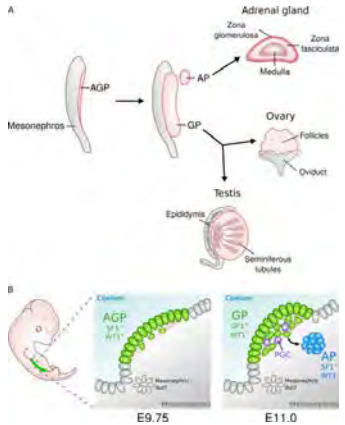
Fig. 1. Diagrammatic representation of gonadal development in mammals. The development of the genital ridge into a testis or ovary proceeds via a series of differentiation steps (heavy arrows). During the indifferent gonad stage, differentiation steps are considered common to both males and females. After the sex-determining event, differentiation diverges and a series of male specific steps leads to testicular development.



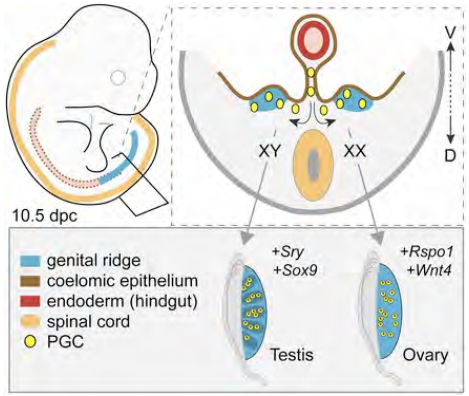








Adrenogonadal primordium (AGP) development in the mouse. (A and B) Schematic representations of AGP differentiation into gonadal primordium (GP) and adrenal primordium (AP).



The establishment of the bipotential genital ridges and gonadal sex determination. In mammals, the genital ridges (blue) typically appear as longitudinal outgrowths along the surfaces of the mesonephroi within the coelomic cavity. In mice, they emerge at ~10 dpc through recruitment of cells from the overlying coelomic epithelium (brown). Primordial germ cells (yellow) colonize the genital ridges (arrows) after leaving the hindgut (red) via the dorsal mesentery. At this stage in development, the genital ridges are bipotential and can differentiate into testes or ovaries, depending on genetic cues. From ~10.5 dpc, the Y-linked sex-determining gene *Sry* is expressed in XY genital ridges and initiates *Sox9* expression and testis differentiation. In the absence of *Sry*, as in XX genital ridges, ovary differentiation is initiated by the action of genes such as *Rspo1* and *Wnt4*. (D) Dorsal; (V) ventral.

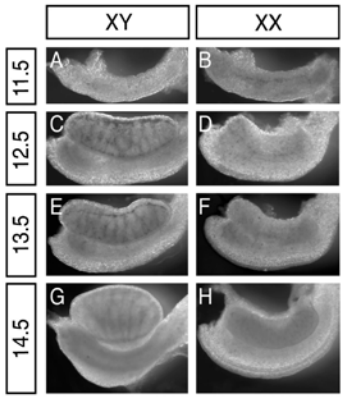


FIGURE 1. Morphological differentiation of mouse embryonic gonads. Whole-mount images of dissected mouse fetal gonads. **A,C,E,G,** XY gonads; **B,D,F,H,** XX gonads. **A,B,** 11.5 dpc; **C,D,** 12.5 dpc; **E,F,** 13.5 dpc; **G,H,** 14.5 dpc.

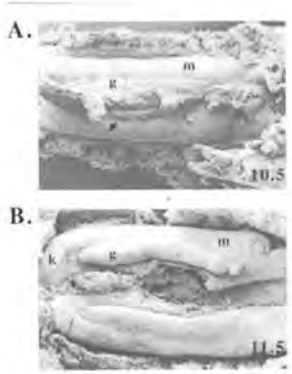


Fig. 2. Scanning electron micrographs of the urogenital ridge. (A) At 10.5 d.p.c. the urogenital ridge fills most of the body cavity between the limb buds. m, mesonephros; g, genital ridge region; the arrow indicates a pore on the coelomic surface of the genital ridge region. (B) By 11.5 d.p.c. the gonad (g) in the mesonephric region of the ridge and kidney (k) in the metanephric region of the ridge are distinct from the mesonephros. (m) No sexual dimorphisms are apparent. Modified from Fig. 8, Capel and Lovell-Badge (1992). Reproduced by permission from the Publishers (JAI Press).

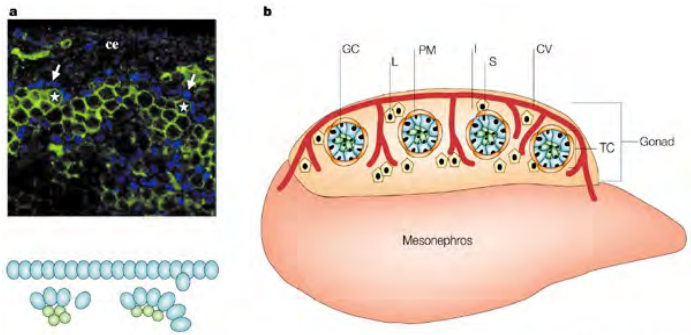
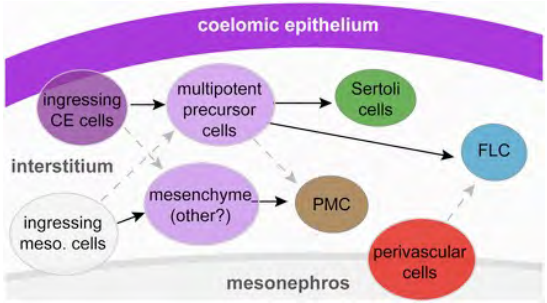


Figure 2 | **Compartmentalization of the testis.** **a** | At the earliest stages of testis organogenesis (11.75–12.0 days post coitum; dpc), Sertoli cells (stained with SF1 antibody; blue) polarize and begin to aggregate around clusters of primordial germ cells (stained with PECAM antibody; asterisk) to initiate development of testis cords. ce, coelomic epithelium. **b** | Between 11.5–12.5 dpc, the cells of the testis are organized into two functional compartments: testis cords (TC) and the interstitial space (I) outside the cords. Within testis cords, Sertoli cells (S; blue) surround germ cells (GC; green). A basal lamina is deposited between Sertoli cells and peritubular myoid cells (PM). The interstitial compartment contains Leydig cells (L; yellow) and the coelomic vessel (CV; red), with branches that extend between cords.



Known and proposed origins of the testicular cell lineages. The cells of the nascent genital ridges originate primarily from the overlying coelomic epithelium but also from the subjacent mesonephros. A subset of ingressing coelomic epithelial cells differentiates into Sertoli cells following *Sry* expression. Some of these supporting cells are also believed to differentiate into FLCs. It is unclear whether cells originating from the mesonephros contribute toward somatic cells other than blood endothelium, but they very likely contribute to the mesenchyme. The origin of PMCs remains unknown, but it is likely that they differentiate from a subset of mesenchymal cells or yet unidentified precursor cells of the testis interstitium. A second origin for the FLCs has also been proposed to include perivascular cells located at the gonad–mesonephric junction.

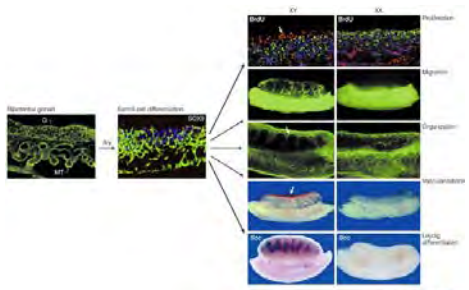
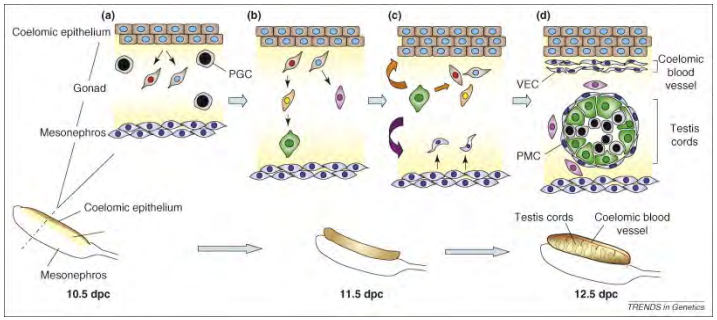
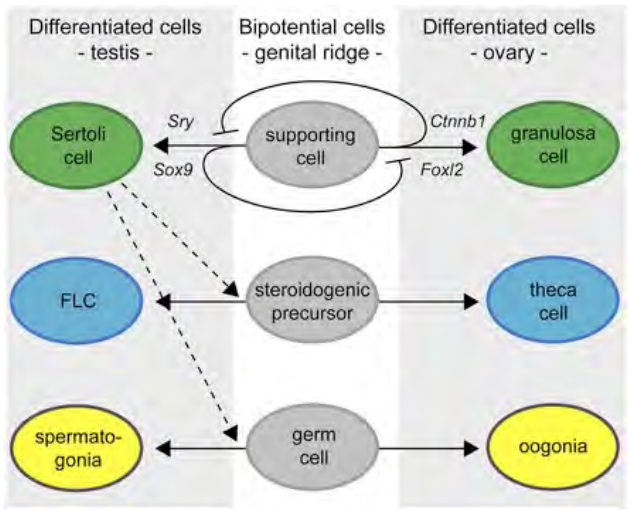
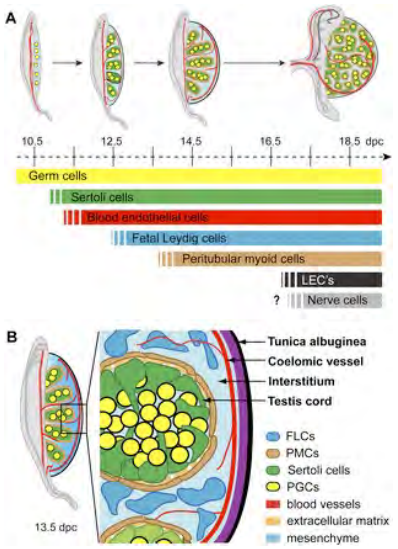


Figure 4 | **Cellular events downstream of *Sry* rapidly organize testis structure.** At the bipotential stage (10.5–11.5 days post coitum; dpc), no obvious morphological features distinguish XX and XY gonads. Antibodies against laminin (green) outline all cells in the gonad (G) and also label the basal lamina of mesonephric tubules (MT) in XX and XY samples. In XY gonads, *Sry* upregulates nuclear SOX9 (blue) in pre-Sertoli cells, and initiates Sertoli-cell differentiation by 11.5 dpc (germ cells and vasculature are labelled with platelet endothelial cell adhesion molecule (PECAM); green). Between 11.5–12.5 dpc, male-specific pathways activate marked morphological and cellular changes in the XY gonad (left column) that do not occur in the XX gonad (right column). These include an upregulation of proliferation in coelomic epithelial cells (measured by BrdU incorporation; red, arrow); migration of cells from the mesonephros (detected in recombinant cultures between a wild-type gonad and a mesonephros in which all cells express GFP; green); structural organization of testis cords (detected by laminin deposition; green); male-specific vascularization (red; blood cells are visible in the light microscope; arrow); and Leydig-cell differentiation (detected by RNA *in situ* hybridization for the steroid enzyme, *Scc*). BrdU image pair repoduced with permission from Ref. 29 © (2000) The Company of Biologists Ltd. XY migration image and vascular image pairs reproduced with permission from Ref. 77 © (2002) Elsevier Science.







GENES REQUIRED TO OBTAIN BIPOTENTIAL GONAD

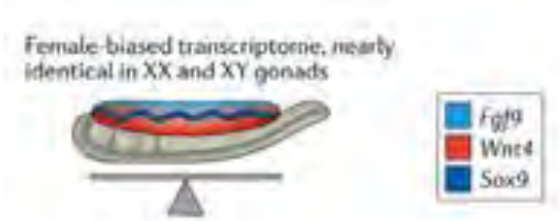
- Found with knockout mice or mutant human tissues not having gonad form from genital ridge.

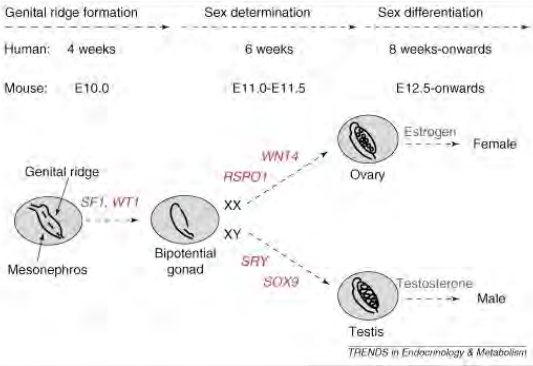
WT1 - Wilms' Tumor, WAGR Syndrome, Frasier Syndrome, Denys-Drash Syndrome
- sex reversal/ different pathologies
- four zinc finger domains
- 16 different products from gene, 11 p13

SF1- Steroidogenic Factor 1, orphan nuclear steroid receptor
- knockout cause lack gonad
- mutation cause sex reversal
- influence MIS and DAX-1 expression

LIM1 - LIM Homeobox gene Lhx9
- knockout cause lack gonad
- LIM knockout cause lack SF1 (? Upstream)

Vertebrate sex determination: evolutionary plasticity of a fundamental switch.
Capel B.
Nat Rev Genet. 2017 Nov;18(11):675-689.





BIOLOGY OF REPRODUCTION **62,** 132–142 (2000)

Role of Neurotropins in Rat Embryonic Testis Morphogenesis (Cord Formation)[1]

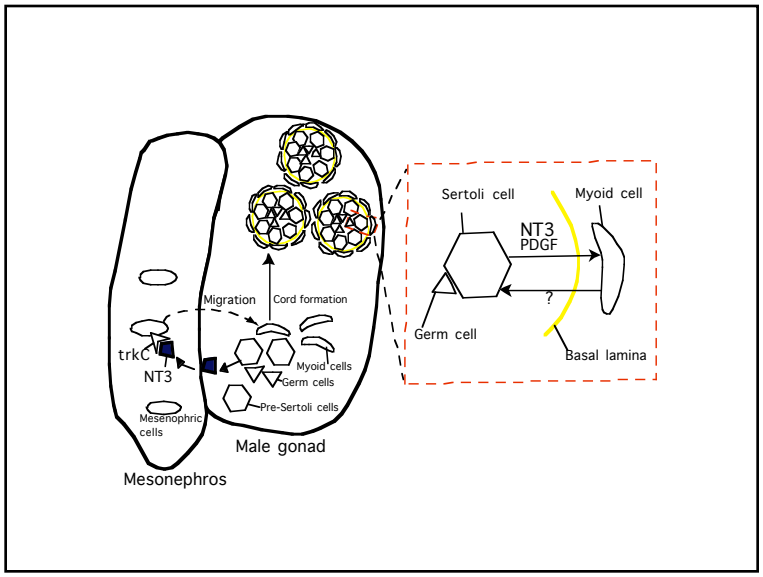
Elena Levine,[3,4] Andrea S. Cupp,[4] and Michael K. Skinner[2]

Center for Reproductive Biology, Department of Genetics and Cell Biology, Washington State University, Pullman, Washington 99164-4231

ABSTRACT

The process of seminiferous cord formation is the first morphological event that differentiates a testis from an ovary and indicates male sex determination. Cord formation occurs by embryonic Day 14 (Day 0 = plug date; E14) in the rat. A series of experiments were conducted to determine if neurotropins and their receptors are important for the process of rat embryonic cord formation. The expression of low affinity neurotropin receptor (p75/LNGFR) was determined by immunohistochemistry on sections of both testis and ovary from E13 through birth (Day 0, P0) with an antibody to p75/LNGFR. The staining for p75/LNGFR was present in the mesonephros of E13 gonads and in a sex-specific manner appeared around developing cords at E14 in the embryonic testis. At birth, staining for p75/LNGFR was localized to a single layer of cells (i.e., peritubular cells) that

amined to determine if there were any morphological differences in the testis. NT3 knockouts appeared to have normal cord morphology in E15 and E17 testis. TrkC knockout mice also had normal cord morphology in E14 and P0 testis. Both NT3 and trkC knockout-mice testis had less interstitial area than wild-type controls. In addition, the trkC knockout mice have an increased number of cells expressing p75LNGFR within the cords when compared to controls or NT3 knockout mice. Combined observations suggest compensation between the different neurotropin ligands, receptors, and/or possibly different growth factors for this critical biological process. In summary, results suggest a novel nonneuronal role for neurotropins in the process of cord formation during embryonic rat testis development. The hypothesis developed is that neurotropins are involved in the progression of male sex differentiation and are critical for the induction of embryonic testis cord formation.



Male-to-Female Sex Reversal in Mice Lacking Fibroblast Growth Factor 9

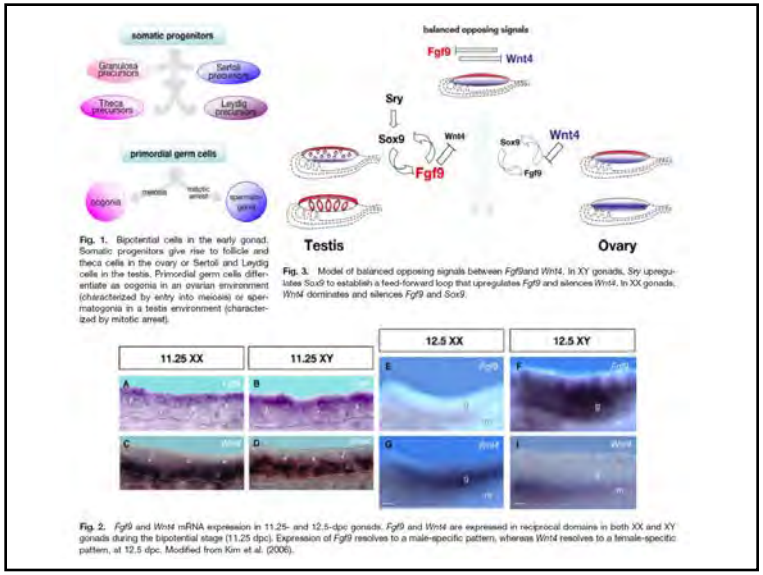
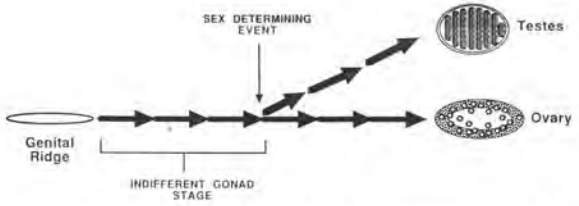


Fig. 1. Bipotential cells in the early gonad. Somatic progenitors give rise to follicle and theca cells in the ovary or Sertoli and Leydig cells in the testis. Primordial germ cells differentiate as oogonia in an ovarian environment (characterized by entry into meiosis) or spermatogonia in a testis environment (characterized by mitotic arrest).

Fig. 3. Model of balanced opposing signals between *Fgf9* and *Wnt4*. In XY gonads, *Sry* upregulates *Sox9* to establish a feed-forward loop that upregulates *Fgf9* and silences *Wnt4*. In XX gonads, *Wnt4* dominates and silences *Fgf9* and *Sox9*.

Fig. 2. *Fgf9* and *Wnt4* mRNA expression in 11.25- and 12.5-dpc gonads. *Fgf9* and *Wnt4* are expressed in reciprocal domains in both XX and XY gonads during the bipotential stage (11.25 dpc). Expression of *Fgf9* resolves to a male-specific pattern, whereas *Wnt4* resolves to a female-specific pattern, at 12.5 dpc. Modified from Kim et al. (2006).



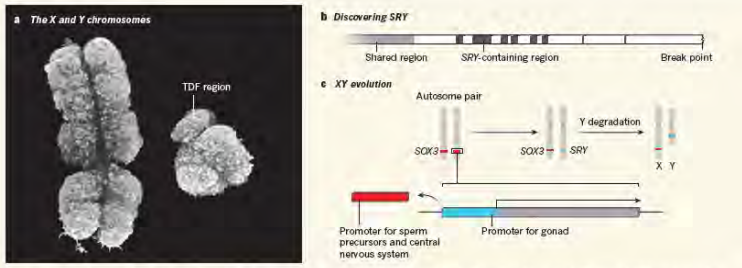
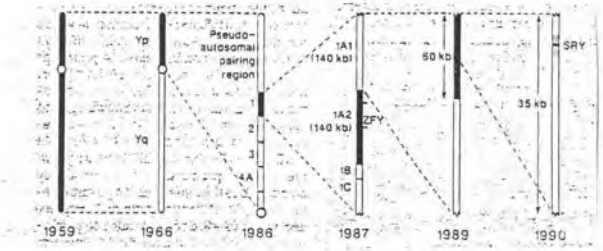
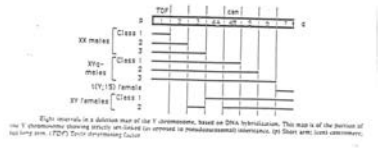
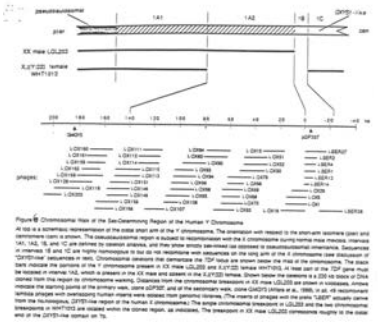
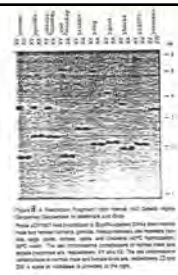


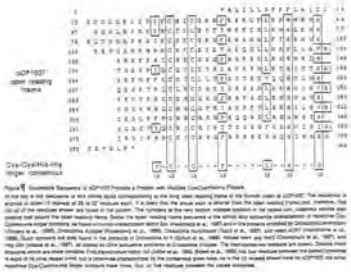
Figure 1 | An evolving understanding of sex. a, In humans, sex is based on the presence or absence of the Y chromosome, seen here with its larger partner, X. The testis-determining factor (TDF) that drives male development was known to lie on the short arm of Y, but its identity was a mystery. b, In 1990, Sinclair *et al.*[1] found two males with only a small piece of Y, which had been broken and fused to the X. They scoured the 35,000 base pairs between the break points and the region at the tip of the Y that is shared with the X, finding several regions (black) that were specific to the Y. One of these regions contained the TDF gene, *SRY*. c, This discovery led to an understanding of how X and Y evolved. The gene *SOX3* was located on a pair of non-sex chromosomes (autosomes) in the ancestors of mammals. A promoter sequence drove expression of *SOX3* in sperm precursors and the central nervous system. The promoter on one copy of *SOX3* was replaced with a sequence that drives expression in the undifferentiated gonad (a tissue that can develop into either an ovary or a testis). This expression pattern allowed the new gene, *SRY*, to direct testis development. Over time, genes not needed for male development were degraded on this chromosome, giving rise to the Y. (Part b adapted from ref. 1.)











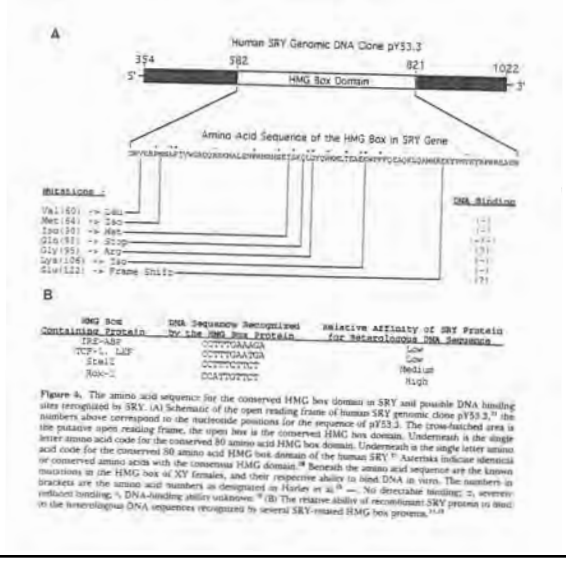
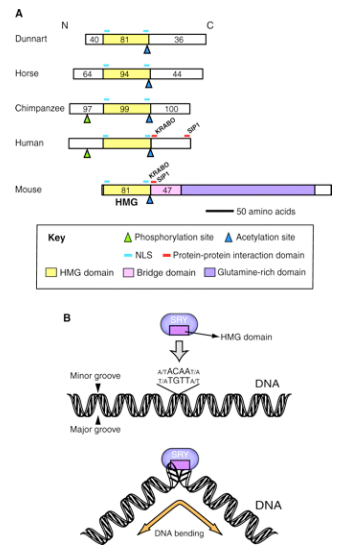


Figure 4. The amino acid sequence for the conserved HMG box domain in SRY and possible DNA binding sites recognized by SRY. (A) Schematic of the open reading frame of human SRY genomic clone pY53.3,[7] the numbers above correspond to the nucleotide positions for the sequence of pY53.3. The cross-hatched area is the putative open reading frame, the open box is the conserved HMG box domain. Underneath is the single letter amino acid code for the conserved 80 amino acid HMG box domain. Underneath is the single letter amino acid code for the conserved 80 amino acid HMG box domain of the human SRY.[8] Asterisks indicate identical or conserved amino acids with the consensus HMG domain.[9] Beneath the amino acid sequence are the known mutations in the HMG box of XY females, and their respective ability to bind DNA in vitro. The numbers in brackets are the amino acid numbers as designated in Harley et al.[10] —, No detectable binding; ±, severely reduced binding; ?, DNA-binding ability unknown.[9] (B) The relative ability of recombinant SRY protein to bind to the heterologous DNA sequences recognized by several SRY-related HMG box proteins.[11,12]



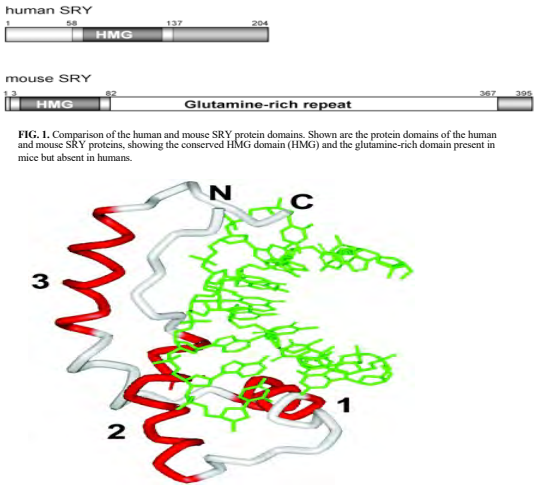


FIG. 1. Comparison of the human and mouse SRY protein domains. Shown are the protein domains of the human and mouse SRY proteins, showing the conserved HMG domain (HMG) and the glutamine-rich domain present in mice but absent in humans.

FIG. 2. A model of the structure of the SRY HMG box bound to DNA. The nuclear magnetic resonance structure of the SRY HMG domain, showing the three α-helices and the L-shaped conformation and binding to the minor groove of DNA causing it to bend and severely unwind (35).

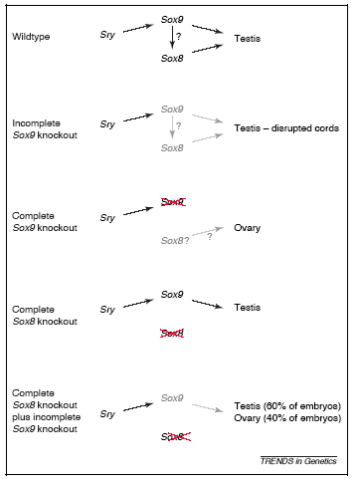
Human SRY Expression at the Sex-determining Period is Insufficient to Drive Testis Development in Mice.

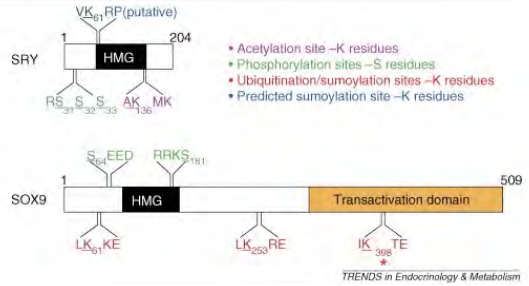
Tsuji-Hosokawa A, Ogawa Y, Tsuchiya I, Terao M, Takada S.

Endocrinology. 2022 Jan 1;163(1):bqab217.

Because the Sry(SRY) XY mice did not develop testis, we concluded that human SRY was insufficient to drive testis development in mouse embryos. The difference in response elements and lack of glutamine-rich domains may have invalidated human SRY function in mice. Signal transduction between Sry/SRY expression and Sox9/SOX9 activation is possibly organized in a species-specific manner.

Sex determination: a tale of two *Sox* genes





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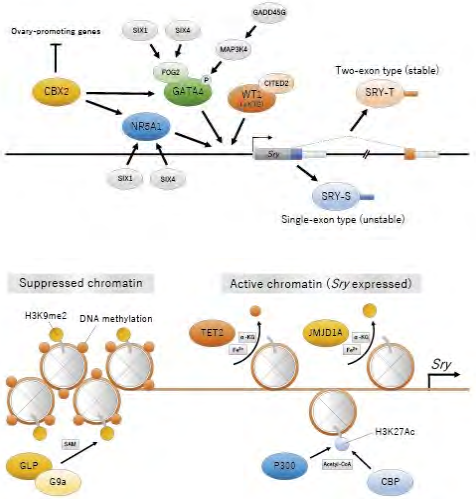
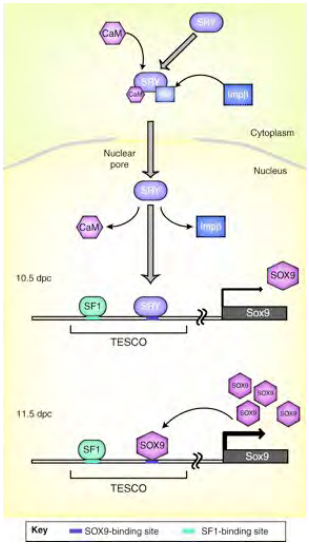
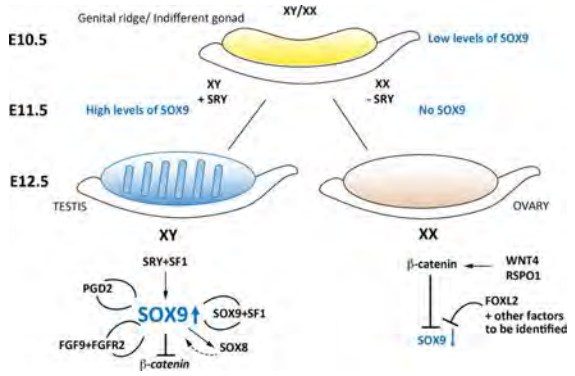
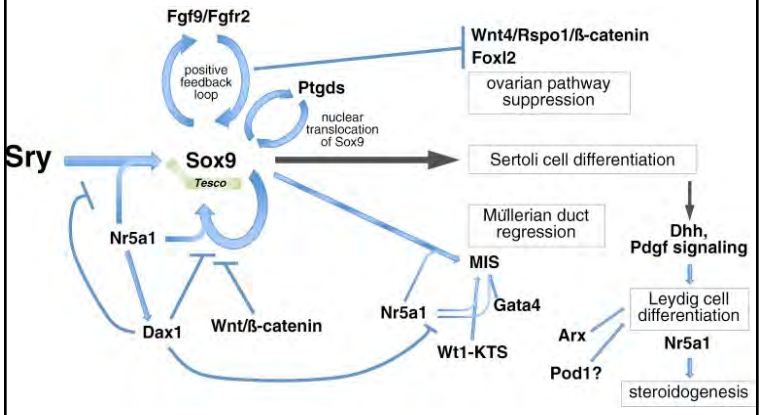
Okashita N, Tachibana M.
Sex Dev. 2021 Sep 28;1-9.





The regulation of Sox9 expression in the gonad.
Gonen N, Lovell-Badge R.
Curr Top Dev Biol. 2019;134:223-252.





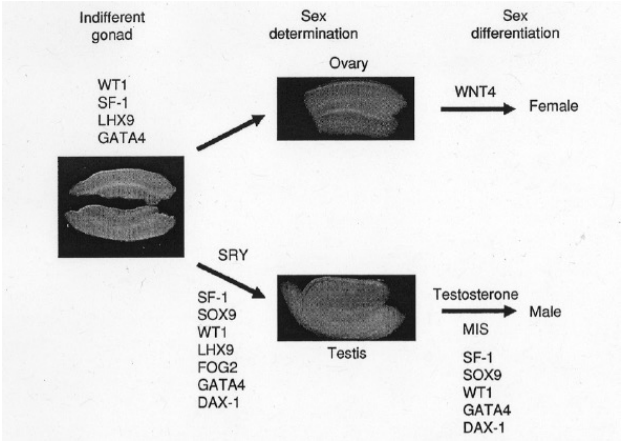
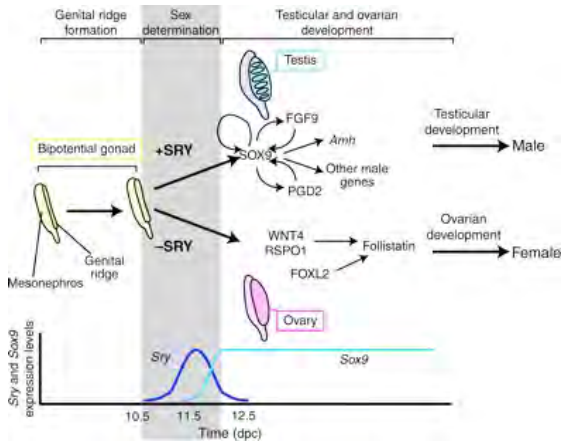
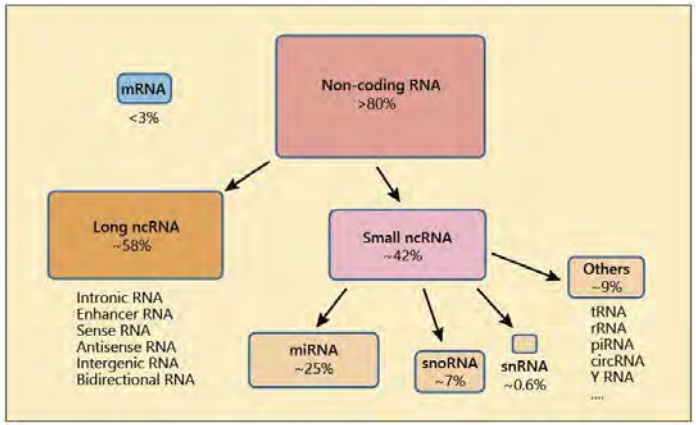


FIGURE 1. Molecular and hormonal determinants of mammalian sex determination and differentiation. The testis (male) and ovary (female) arise from a common precursor, the biopotential or indifferent gonad. The testis-determining factor (SRY) is essential for male gonad development. The other key factors involved in mammalian sex development are also indicated.



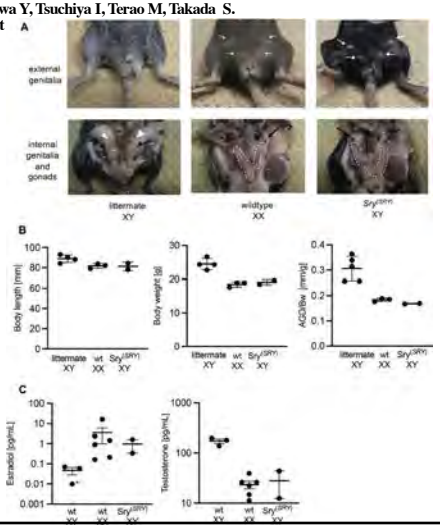
Non-Coding RNAs: lncRNAs, miRNAs, and piRNAs in Sexual Development.
Burgos M, Hurtado A, Jiménez R, Barrionuevo FJ.
Sex Dev. 2021 Oct 6;1-16.



Gene	Human 3' UTR length, bp (TargetScan)	Conserved miRNAs bBinding site in 3' UTR (TargetScan)	Validated target and tissue
SRY	134	–	–
SOX9	2,033	**miR-101-3p**,**miR-138-5p**,**miR-1-3p**/**miR-206**/**miR-613**,**miR-145-5p**/*miR-5195-3p, miR-302-3p/miR-520-3p*,**miR-30-5p**	**miR-101-3p**; lung cancer [Kong et al., 2019b] **miR-138-5p**; hepatocellular carcinoma, renal cell carcinoma [Liu et al., 2016; Hu et al., 2017] **miR-1-3p**; hepatocellular carcinoma, developmental dysplasia of the hip (DHH) [Zhang et al., 2019] **miR-206**; lung cancer, Legg-Calvé-Perthes disease, hepato-cellular carcinoma [Zhang et al., 2015b, 206; Luo et al., 2018; Lin et al., 2021] **miR-613**; glioma, hepatocellular carcinoma, gastric cancer [Li et al., 2019; Sang et al., 2018; Xue et al., 2019] **miR-145-5p**; chondrogenic differentiation, cartilage develop-ment [Yang et al., 2011; Martinez-Sanchez et al., 2012; Rani et al., 2013; Cui et al., 2021;Wang and Yang, 2020] **miR-30-5p**; lung cancer, colon cancer [Zhou et al., 2019; Sun et al., 2020]
FGF9	3,066	**miR-140-5p**,**miR-182-5p** *miR-425-5p, miR-96-5p/miR-1271-5p, miR-302-3p*/**miR-372-3p**/*miR-373-3p/miR-520-3p*,**miR-187-3p**,*miR-499-5p, miR-183-5p, miR-155-5p, miR-140-3p, miR-219-5p/ miR-4782-3p/miR-6766-3p, miR-9-5p, miR-137, miR-15-5p/ miR-16-5p/miR-195-5p/miR-424-5p/miR-497-5p/miR-6838-5p, miR-143-3p/miR-4770/miR-6088*	**miR-140-5p**; hepatocellular carcinoma, laryngeal squamous cell carcinoma, lung cancer, bladder cancer, odontoblastic differentiation [Yang et al., 2013; Wang et al., 2019; Li et al., 2020; Wu et al., 2020b; Zhong et al., 2020] **miR-182-5p**; Schwann cell, hallux valgus deformity [Yu et al., 2012; Zhang et al., 2021] **miR-372-3p**; lung squamous cell carcinoma (LSCC) [Wang et al., 2017] **miR-187-3p**; cervical cancer, non-small-cell lung cancer (NSCLC), breast cancer [Liang et al., 2017; Liang et al., 2020; Wu et al., 2020c]
FOXL2	1,368	*miR-23-3p/miR-130-5p*,**miR-133a-3p.1**, *miR-133a-3p.1/* **miR-133b**,*miR-302-3p/miR-372-3p/miR-373-3p/miR-520-3p, miR-17-5p/miR-20-5p/miR-51/miR-93-5p/miR-106-5p/ miR-519-3p/miR-526-3p*	**miR-133a**; differentiation of C2C12 cells [Luo et al., 2015] **miR-133b**; estrogen production in granulosa cells [Dai et al., 2013]
RSPO1	1,476	*miR-142-5p/miR-5590-3p* *miR-203-3p*	–
DMRT1	951	–	–
WNT4	2,745	*miR-9-5p, miR-204-5p/miR-211-5p, miR-133-3p, miR-24-3p, miR-15-5p/miR-16-5p/miR-195-5p/miR-424-5p/miR-497-5p/miR-6838, miR-103-3p/miR-107*	–
PTGDS	164	–	–

miRNAs whose target has been validated in experimental studies are shown in bold font.

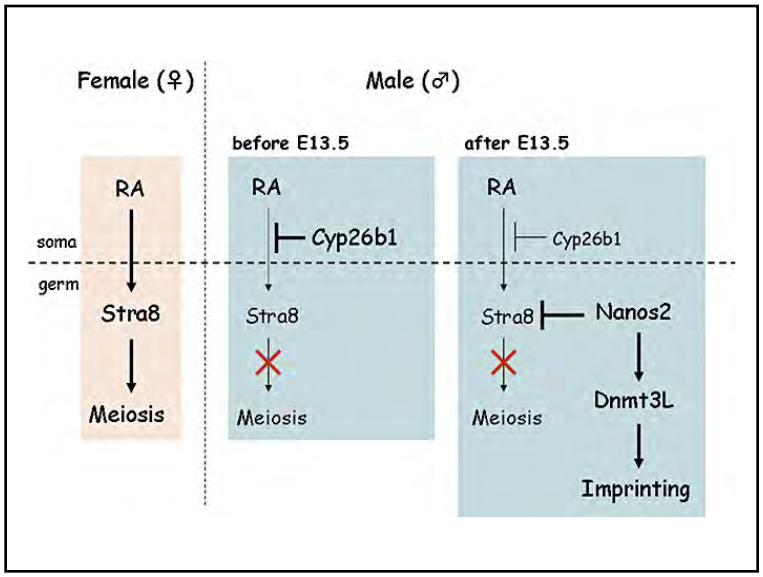
Human SRY expression at the sex-determining period is insufficient to drive testis development in mice.
Tsuji-Hosokawa A, Ogawa Y, Tsuchiya I, Terao M, Takada S.
Endocrinology. 2021 Oct

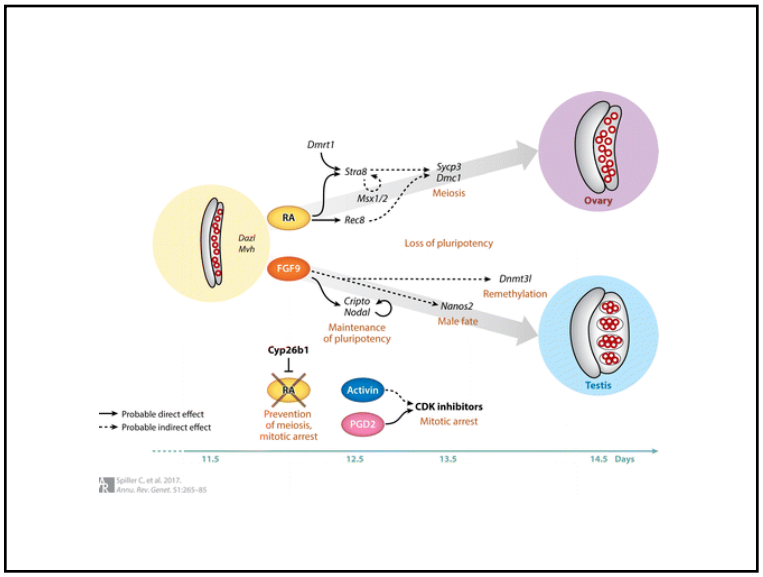


SOX4 regulates gonad morphogenesis and promotes male germ cell differentiation in mice.
Zhao L, Arsenault M, Ng ET, Longmuss E, Chau TC, Hartwig S, Koopman P.
Dev Biol. 2017 Mar 1;423(1):46-56.

Abstract

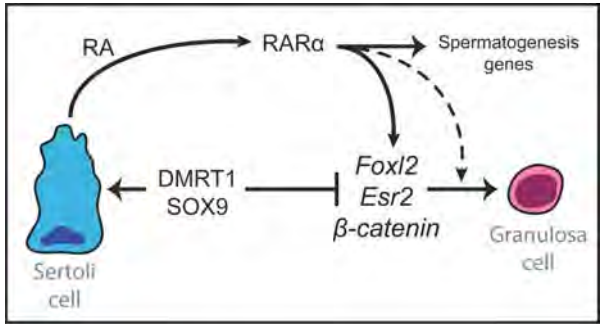
The group C SOX transcription factors SOX4, -11 and -12 play important and mutually overlapping roles in development of a number of organs. Here, we examined the role of SoxC genes during gonadal development in mice. All three genes were expressed in developing gonads of both sexes, predominantly in somatic cells, with Sox4 being most strongly expressed. Sox4 deficiency resulted in elongation of both ovaries and testes, and an increased number of testis cords. While female germ cells entered meiosis normally, male germ cells showed reduced levels of differentiation markers Nanos2 and Dnmt3l and increased levels of pluripotency genes Cripto and Nanog, suggesting that SOX4 may normally act to restrict the pluripotency period of male germ cells and ensure their proper differentiation. Finally, our data reveal that SOX4 (and, to a lesser extent, SOX11 and -12) repressed transcription of the sex-determining gene Sox9 via an upstream testis-specific enhancer core (TESCO) element in fetal gonads, raising the possibility that SOXC proteins may function as transcriptional repressors in a context-dependent manner.





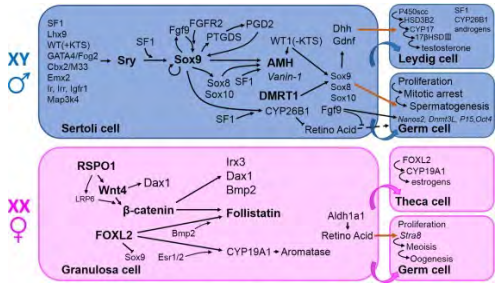
DMRT1 protects male gonadal cells from retinoid-dependent sexual transdifferentiation.

Minkina A, et al. Dev Cell. 2014 Jun 9;29(5):511-20.



Model for role of DMRT1 in sex maintenance

DMRT1 directly represses transcription in Sertoli cells of potential feminizing genes including *Foxl2, Esr2*, and the *Wnt/β-catenin* pathway genes *Wnt4* and *Rspo1* (Matson et al., 2011). This paper shows that DMRT1 thereby allows Sertoli cells to produce RA that is necessary for spermatogenesis without causing RARα to activate these feminizing genes, which also activate one another. The model also indicates that it is possible, based on data from other systems, that RARα synergizes with products of some of the feminizing genes to drive transdifferentiation. In addition to the genes shown, DMRT1 also represses *Cyp19a1/aromatase*, which makes estradiol that stimulates ER activity (Matson et al., 2011).



Molecular models of gene regulatory networks in mammalian sex determination and gonadal development. During sex determination, SF1, Lhx9, WT1 (+KTS), GATA4/Fog2, Cbx2/M33, Emx2, Ir, Irr, Igfr1 and Map3k4 are expressed in the undifferentiated male gonads and are essential for Sry expression. Sry and SF1 directly regulates Sox9 expression, and then triggers a cascade of genes to mediate testis development. Sox9 maintains its own expression through Sox9/SF1, Fgf9/FGFR2 and PTGDS/PGD2-mediated pathways. Sox9 then regulates the expression of Amh, Vanin-1 and Cyp26b1. In the female gonad, Rspo1 and Wnt4 regulate the β-catenin pathway to regulate the expression of follisatin and establish female ovarian development. Foxl2, synergizes with Esr1/2 to regulate the expression of Cyp19a1. This finally produces aromatase in the granulosa cells and estrogens in theca cells. Retinoic acid, a product of Aldh1a1, is essential for the expression of Stra8 in female germ cells and initiates meiosis and oogenesis. However, in male gonads, Cyp26b1 and Fgf9 signals antagonize the functions of retinoic acid, induce the mitotic arrest (G0/G1 arrest) of male germ cells and are responsible for the expression of several male germ cell markers (Nanos, Dnmt3L, P15, Oct4). After the sex determination stage, Sox8, Sox9 and Sox10 are required for the maintenance of male fertility and the expression of Dhh and Gdnf, which then induces testosterone secretion in Leydig cells.

TABLE 1 Genes in mammalian sex determination and early gonadal differentiation known at the year indicated

1990	*1995*	*2001*
SRY	*SRY*	*SRY*
	WT1	*WT1*
	SF1	*SF1*
	DAX1	*DAX1*
	SOX9	*SOX9*
	LHX1(LIM1)	*LHX1(LIM1)*
		EMX2
		DMRT1
		M33
		GATA4
		LHX9
		VNN1
		FGF9
		WNT4

Genes are listed chronologically, in the order of their first implication in sex determination during the time intervals 1991–1995 and 1996–2001. For references, see Koopman (2001) and text.

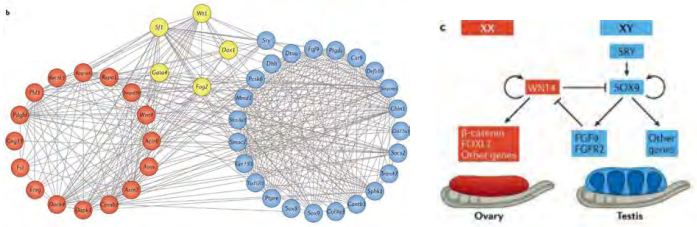


Table 1 Genes involved in the male and female sex determination pathway

Gene	Protein function	Gain- and loss-of-function phenotypes: Human syndrome	Gain- and loss-of-function phenotypes: Mouse models	References
Genes involved in initial development the bipotential gonad				
Emx2	Transcription factor	–	Aberrant tight junction assembly, failure in genital ridge formation (LOF)	Kusaka *et al.* (2010)
Gata4	Transcription factor	Ambiguous external genitalia, congenital heart disease (LOF)	Failure in thickening of the coelomic epithelium, defective initial formation of genital ridge (LOF)	Lourenço *et al.* (2011), Manuylov *et al.* (2011) and Hu *et al.* (2013)
Wt1	Transcription factor	Denys-Drash, Frasier syndrome (LOF)	Disruption of seminiferous tubule and somatic cell apoptosis, XY sex reversal (LOF)	Kreidberg *et al.* (1993), Hammes *et al.* (2001) and Gao *et al.* (2006)
Lhx9	Transcription factor	–	Failure in genital ridge formation (LOF)	Birk *et al.* (2000)
Sf1	Nuclear receptor	Embryonic testicular regression syndrome, gonadal dysgenesis	Delayed organization of male testis cord, failure in genital ridge formation (LOF)	Park *et al.* (2005) and Lin *et al.* (2007)
Genes involved in the regulation of SRY expression during primary sex determination				
Gata4/Fog2	Transcription/cofactor	–	Apparent XY gonadal sex reversal (LOF)	Tevosian *et al.* (2002)
Gadd45γ	Stress-response protein	–	XY sex reversal (LOF)	Gierl *et al.* (2012) and Warr *et al.* (2012)
Map3k4	Kinase	–	XY sex reversal (LOF)	Bogani *et al.* (2009)
Cbx2	Transcription factor	XY ovarian DSD, XY sex reversal (LOF)	XY sex reversal (LOF)	Biason-Lauber *et al.* (2009) and Katoh-Fukui *et al.* (2012)
Ir, Irr, Igf1r	Membrane receptor	–	XY sex reversal (LOF)	Nef *et al.* (2003)
Genes involved in male testis determination pathway				
SRY	Transcription factor	Turner syndrome, Klinefelter syndrome, XY sex reversal (LOF)	XY sex reversal (LOF); XX sex reversal (GOF)	Ford *et al.* (1959), Jacobs & Strong (1959), Koopman *et al.* (1989) and Gubbay *et al.* (1990)
Sox9	Transcription factor	Campomelic dysplasia XY sex reversal (LOF)	Abnormal Sertoli cell differentiation, XY sex reversal (LOF); XX sex reversal (GOF)	Foster *et al.* (1994), Huang *et al.* (1999), Vidal *et al.* (2001), Chaboissier *et al.* (2004) and Barrionuevo *et al.* (2006)
Amh	Hormone	XY sex reversal (LOF)	XY sex reversal (LOF)	
Fgf9	Growth factor	XY sex reversal (LOF)	XY sex reversal (LOF)	Kim *et al.* (2006) and Jameson *et al.* (2012)
Dmrt1	Transcription factor	XY gonadal dysgenesis, XY sex reversal (LOF)	Postnatal feminization in XY mice, defective seminiferous tubule (LOF)	Matson *et al.* (2012)

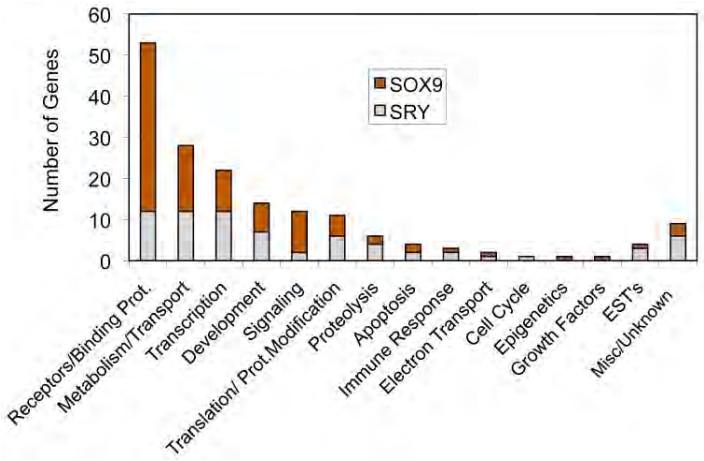
Table 1 *Continued*

Gene	Protein function	Gain- and loss-of-function phenotypes: Human syndrome	Gain- and loss-of-function phenotypes: Mouse models	References
Genes involved in female ovary determination pathway				
Wnt4	Signaling molecule	Ambiguous genitalia (GOF)	Failure in the formation of coelomic vessel and germ cell, degeneration of the female reproductive tract, partial XX sex reversal (LOF)	Jordan *et al.* (2003) and Yao *et al.* (2004)
β-catenin	Transcription factor	–	Partial XX sex reversal (LOF)	Chassot *et al.* (2008)
Rspo1	Growth factor	Palmoplantar hyperkeratosis, skin carcinoma, complete XX sex reversal (LOF)	Development of ovotestes, partial XX sex reversal (LOF)	Parma *et al.* (2006) and Chassot *et al.* (2008)
Foxl2	Transcription factor	BPES (LOF)	Premature ovarian failure, ablation of the primordial follicle pool, partial XX sex reversal (LOF)	Uhlenhaut & Treier (2006) and Ottolenghi *et al.* (2007)
Dax1	Nuclear receptor	XX sex reversal (LOF)	XX sex reversal (LOF)	Swain *et al.* (1998) and Meeks *et al.* (2003)

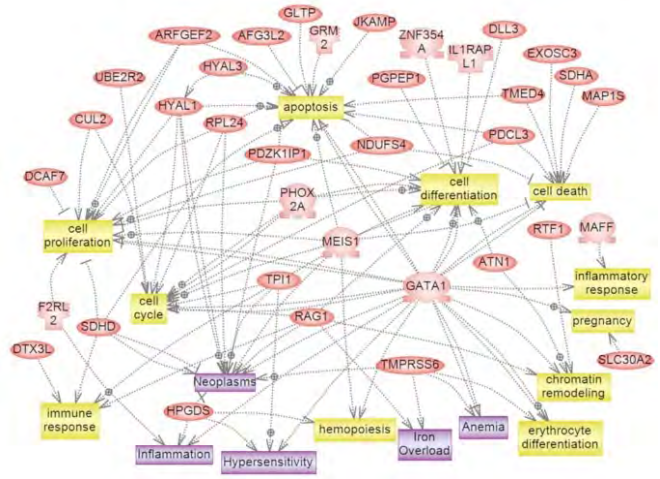
–, no mutations have been identified to date in human sexual reversal patients; GOF, gain of function; LOF, loss of function; BPES, blepharophimosis/ptosis/epicanthus inversus syndrome.

Bhandari RK, Haque MM, Skinner MK. (2012) Global genome analysis of the downstream binding targets of testis determining factor SRY and SOX9. PLoS One. 7(9):e43380.

A major event in mammalian male sex determination is the induction of the testis determining factor Sry and its downstream gene Sox9. The current study provides one of the first genome wide analyses of the downstream gene binding targets for SRY and SOX9 to help elucidate the molecular control of Sertoli cell differentiation and testis development. A modified ChIP-Chip analysis using a comparative hybridization was used to **identify 71 direct downstream binding targets for SRY and 109 binding targets for SOX9**. Interestingly, only 5 gene targets overlapped between SRY and SOX9. In addition to the direct response element binding gene targets, a large number of atypical binding gene targets were identified for both SRY and SOX9. Bioinformatic analysis of the downstream binding targets identified gene networks and cellular pathways potentially involved in the induction of Sertoli cell differentiation and testis development. The specific DNA sequence binding site motifs for both SRY and SOX9 were identified. Observations provide insights into the molecular control of male gonadal sex determination.

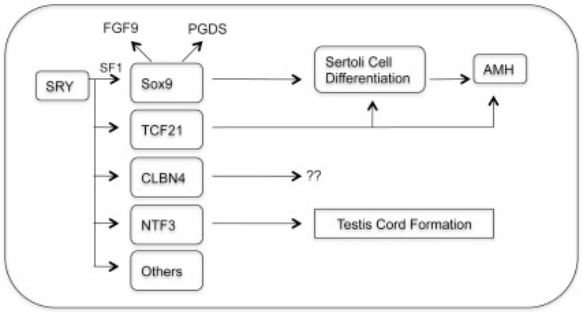


SRY direct binding target genes and their association to functional cellular processes



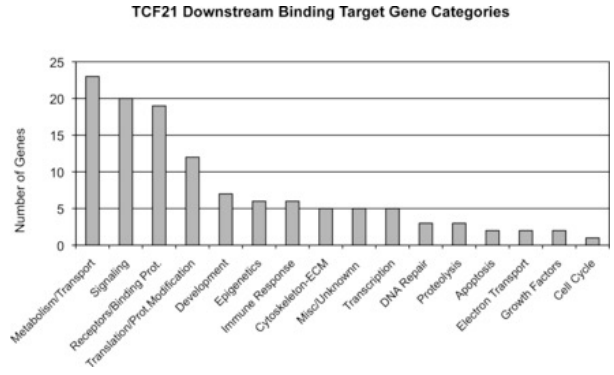
Bhandari RK, Sadler-Riggleman I, Clement TM, Skinner MK. (2011) Basic helix-loop-helix transcription factor TCF21 is a downstream target of the male sex determining gene SRY. PLoS One. 6(5):e19935.

The cascade of molecular events involved in mammalian sex determination has been shown to involve the SRY gene, but specific downstream events have eluded researchers for decades. The current study identifies one of the first direct downstream targets of the male sex determining factor SRY as the basic-helix-loop-helix (bHLH) transcription factor TCF21. SRY was found to bind to the Tcf21 promoter and activate gene expression. Mutagenesis of SRY/SOX9 response elements in the Tcf21 promoter eliminated the actions of SRY. SRY was found to directly associate with the Tcf21 promoter SRY/SOX9 response elements in vivo during fetal rat testis development. TCF21 was found to promote an in vitro sex reversal of embryonic ovarian cells to induce precursor Sertoli cell differentiation. TCF21 and SRY had similar effects on the in vitro sex reversal gonadal cell transcriptomes. Therefore, SRY acts directly on the Tcf21 promoter to in part initiate a cascade of events associated with Sertoli cell differentiation and embryonic testis development.

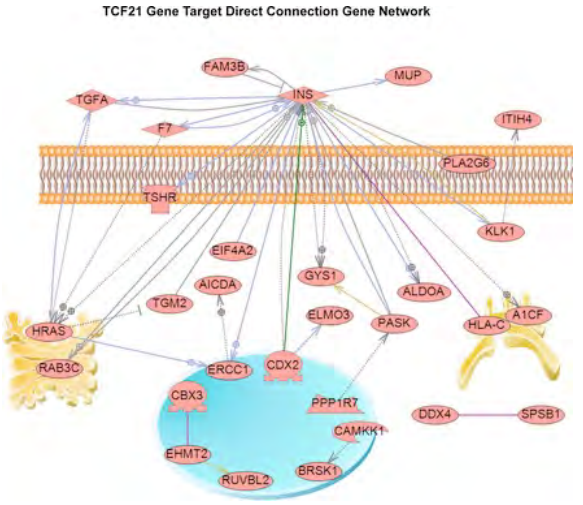


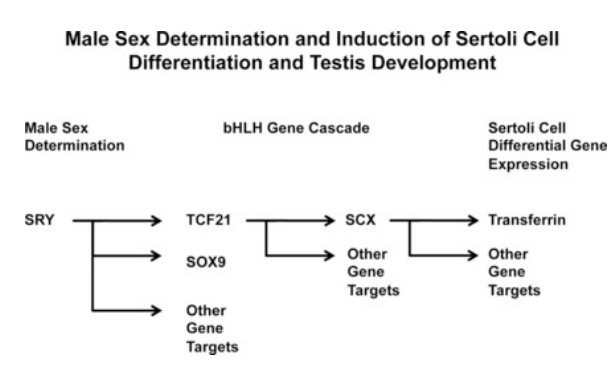
Summary of SRY downstream genes.
Proposed downstream actions of SRY on *Sox9* and *Tcf21* genes, along with *Clbn4*, *Ntf3*, and others yet to be identified. TCF21 induction of Sertoli cell differentiation and expression of marker genes such as *Amh* indicated. Combined actions of SRY and SF1 on *Sox9* expression and actions on *Fgf9* and *Pgds* expression indicated.

Bhandari RK, Schinke EN, Haque MM, Sadler-Riggleman I, Skinner MK. (2012) SRY induced TCF21 genome-wide targets and cascade of bHLH factors during Sertoli cell differentiation and male sex determination in rats. Biol Reprod. 87(6):131.



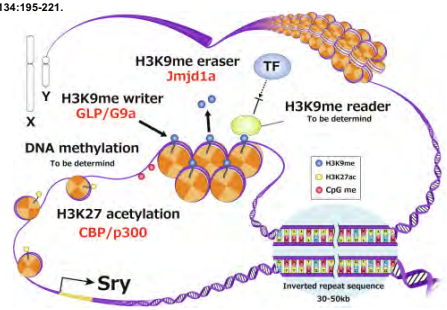
TCF21 binding target gene functional categories. Total numbers of target genes associated with a specific category are presented on the y-axis and gene functional categories on the x-axis.





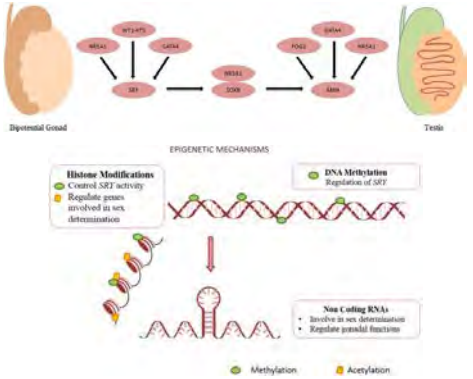
Schematic diagram of the hypothesized cascade of bHLH transcription factors involved in Sertoli cell differentiation and gonadal sex determination.

Role of epigenetic regulation in mammalian sex determination.
Miyawaki S, Tachibana M.
Curr Top Dev Biol. 2019;134:195-221.

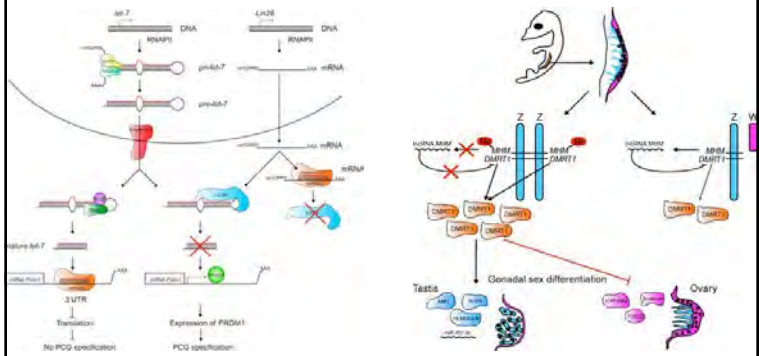


The contributors for the epigenetic regulation of Sry. Sry gene is surrounded by large inverted repeats located in the male-specific region of the Y chromosome. Epigenetic modifications such as DNA methylation, histone methylation, and histone acetylation can alter the accessibility of DNA to transcription machinery and therefore influence gene expression. The epigenetic regulation is achieved by functional coupling between the enzymes that modify chromatin (writers), proteins that bind to specific modification and trigger the downstream pathway (readers), and the enzymes that remove modifications (erasers). In recent years, it has been revealed that multiple epigenetic regulatory mechanisms are involved in ensuring the accurate expression of Sry. Jmjd1a and GLP/G9a complex are the enzymes that demethylate and methylate H3K9 at the Sry locus. The reader protein that recognizes H3K9me at the Sry locus has not been identified. The histone acetyltransferases p300/CBP targets H3K27ac at the Sry locus. DNA demethylation concomitantly occurs with Sry activation. However, DNA demethylation pathway of the Sry locus should be determined. TF: Transcription factor.

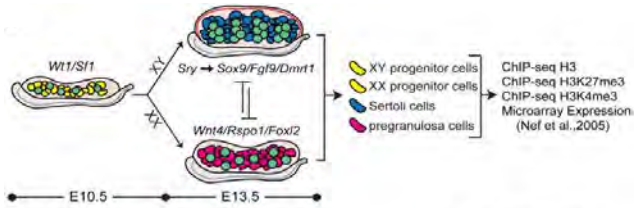
Genetic and epigenetic effects in sex determination.
Gunes SO, Metin Mahmutoglu A, Agarwal A.
Birth Defects Res C Embryo Today. 2016 Dec;108(4):321-336.

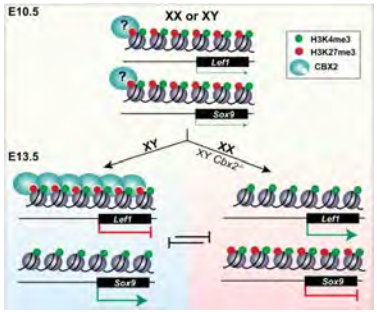


The role of non-coding RNAs in male sex determination and differentiation.
Rastetter RH, Smith CA, Wilhelm D.
Reproduction. 2015 Sep;150(3):R93-107.



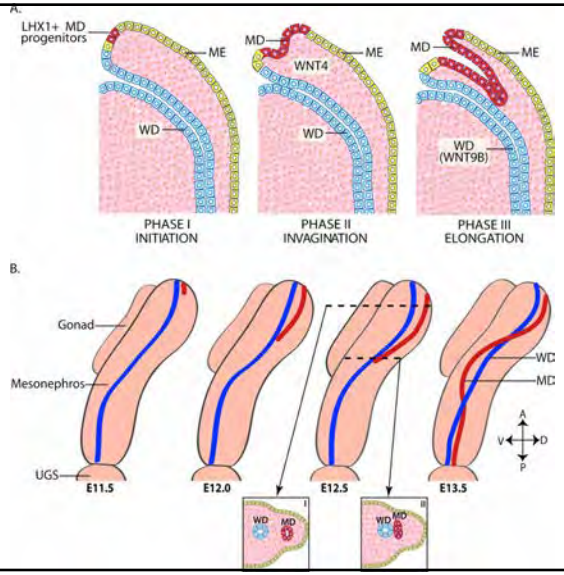
CBX2 is required to stabilize the testis pathway by repressing Wnt signaling.
Garcia-Moreno SA, Lin YT, Futtner CR, Salamone IM, Capel B, Maatouk DM.
PLoS Genet. 2019 May 22;15(5):e1007895.

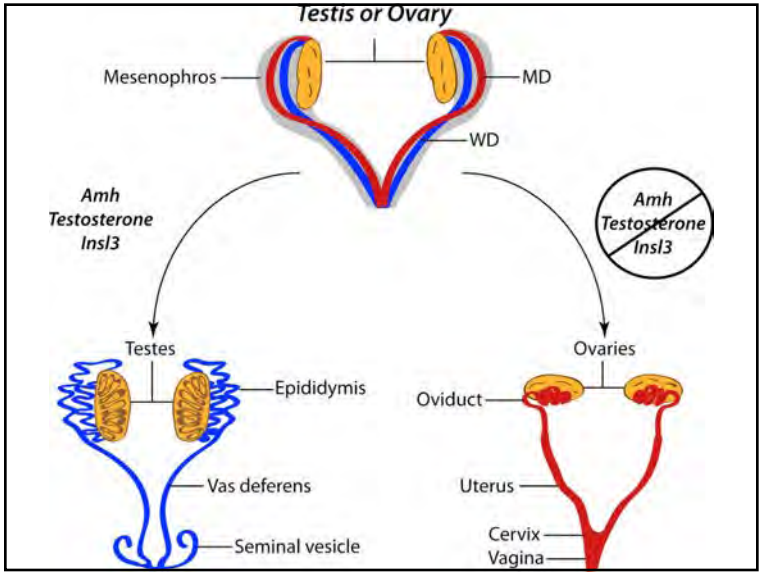




Model of the epigenetic regulation of mammalian sex determination.
At the bipotential stage (E10.5), testis- (eg. Sox9) and ovary-determining (eg. Lef1) genes are bivalent, marked by both H3K27me3 and H3K4me3. Bivalent SD genes are co-expressed at low levels, poised for expression of Sry and commitment to the testis fate (XY, blue) or in absence of Sry, commitment of the ovary fate through the Wnt signaling pathway (XX, pink). Upregulation of SD genes is accompanied by loss of H3K27me3. Genes that promote the alternate fate and are repressed after sex determination (E13.5) remain bivalent. CBX2 binds to Wnt's downstream target Lef1 in XY gonads, inhibiting its upregulation and stabilizing the testis fate. In XX E13.5 gonads, or in XY gonads that lack Cbx2, Lef1 promotes pregranulosa development which blocks upregulation of the testis fate (right, pink). It remains unclear whether CBX2 maintains H3K27me3 from the progenitor state in XY cells and is removed from specific targets in XX cells, or whether it is targeted specifically to ovary genes during Sertoli cell development.

Sex Differentiation





Role Testosterone -

1)Wolffian Duct development

2) Male Reproduction Genitalia

3) External Genitalia

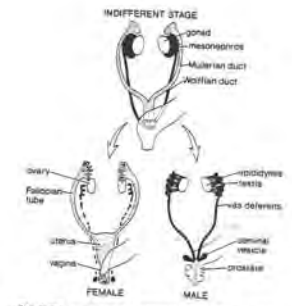


FIG. 7. Differentiation of the internal genitalia. (From ref. 256.)

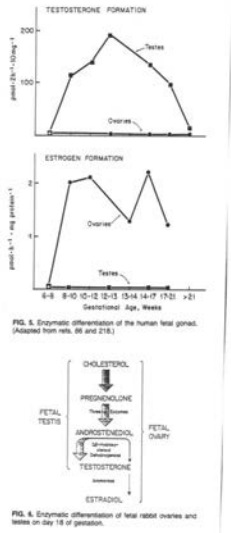


FIG. 5. Enzymatic differentiation of the human fetal gonad. (Adapted from refs. 86 and 218.)

FIG. 6. Enzymatic differentiation of fetal rabbit ovaries and testes on day 18 of gestation.

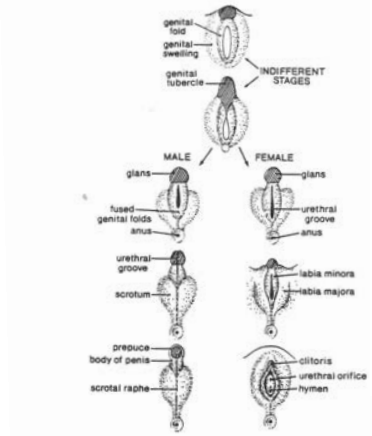


FIG. 8. Differentiation of the external genitalia. (From ref. 256.)

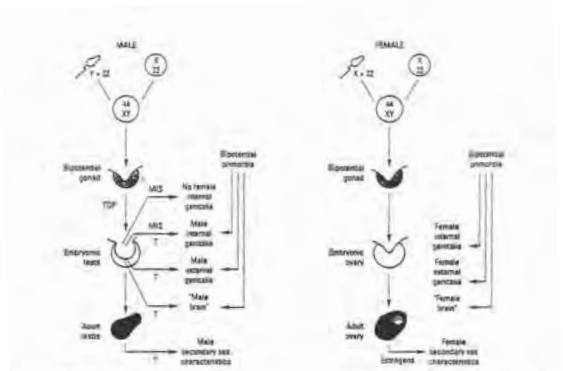
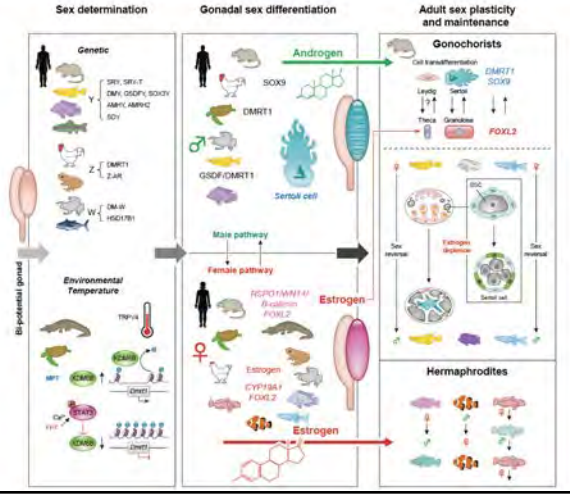


Figure 23-6. Diagrammatic summary of normal sex determination, differentiation, and development in humans. MIS, mullerian inhibiting substance; T, testosterone or other androgen.

Sex Determination in Other Species

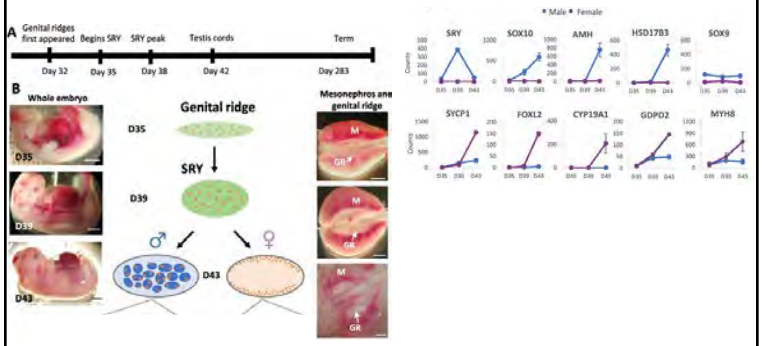
Sex determination, gonadal sex differentiation, and plasticity in vertebrate species.
Nagahama Y, Chakraborty T, Paul-Prasanth B, Ohta K, Nakamura M.
Physiol Rev. 2021 Jul 1;101(3):1237-1308.

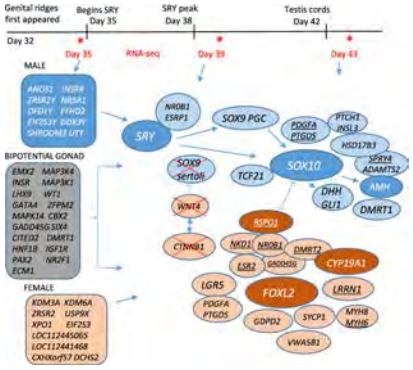


Animal	Species	Genetic type	SD gene	Protein function	Reference
	Mammals	XY/XX	*SRY/Sry*	TF	*Sinclair et al 1990 (104)*
	Medaka, Oryzias latipes	XY/XX	*DMY*	TF	*Matsuda et al 2002 (10)*
	Medaka, Oryzias latipes	XY/XX	*dmrt1by*	TF	*Nanda et al 2002 (121)*
	African clawed frog, Xenopus laevis	ZZ/ZW	*DM-W*	TF	*Yoshimoto et al 2008 (6)*
	Chicken, Gallus gallus domesticus	ZZ/ZW	*DMRT1*	TF	*Smith et al 2009 (13)*
	Patagonian pejerrey, Odontesthes hatcheri	XY/XX	*amhy*	H	*Hattori et al 2012 (2)*
	Luzon medaka, Oryzias luzonensis	XY/XX	*Gsdf*^{Y}	GF	*Myosho et al 2012 (16)*
	Tiger pufferfish, Takifugu rubripes	XY/XX	*Amhr2*	HR	*Kamiya et al 2012 (146)*
	Rainbow trout, Oncorhynchus mykiss	XY/XX	*sdY*	IRF	*Yano et al 2012 (17)*
	Indian medaka, Oryzias dancena	XY/XX	*Sox3*^{Y}	TF	*Takehana et al 2014 (18)*
	Nile tilapia, Oreochromis niloticus	XY/XX	*amhy*	H	*Li et al 2015 (3)*
	Turquoise killifish, Nothobranchius furzeri	XY/XX	*gdf6Y*	GF	*Reichwald et al 2015 (151)*
	Japanese wrinkled frog, Glandirana rugosa	ZZ/ZW	*Z-AR*	HR	*Oike et al 2017 (19)*
	Chinese tongue sole, Cynoglossus semilaevis	ZZ/ZW	*dmrt1*	TF	*Cui et al 2017 (14)*
	Seriola fishes	ZZ/ZW	*Hsd17b1*	SE	*Koyama et al 2019 (155)*
	Northern pike, Esox lucius	XY/XX	*amhbY*	H	*Pan et al 2019 (4)*
	Mouse	XY/XX	*Sry-T*	TF	*Miyawaki et al 2020 (106)*

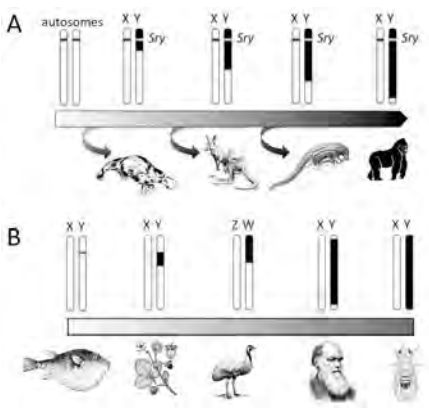
FIGURE 1. Diversity of sex determination systems and master sex determining genes across the vertebrate species. Studies conducted across various vertebrate species have unraveled the diversity in sex chromosomes, sex determination systems, and the master sex-determining switches that control the genetic cascade involved in sex determination (SD). Ranging from humans to Northern pike, discovery of SD genes has helped to understand the vulnerability of sexual reproduction, which is addressed by evolutionary pressures through rapid turnover in sex chromosomes and SD genes to bestow the organisms with superior competence for survival. Identification of master SD genes has also helped us to understand how Nature repurposes the existing resources. Descriptions of each gene found so far from various vertebrates are shown in the chronological order of their discovery from top to bottom. GF, growth factor; H, anti-Müllerian hormone; IRF, interferon regulatory factor; SE, steroidogenic enzyme; SR, steroid receptor; TF, transcription factor.

Gene expression profiles of bovine genital ridges during sex determination and early differentiation of the gonads.
Planells B, Gómez-Redondo I, Sánchez JM, McDonald M, Cánovas Á, Lonergan P, Gutiérrez-Adán A.
Biol Reprod. 2019 Aug 28. [Epub ahead of print]

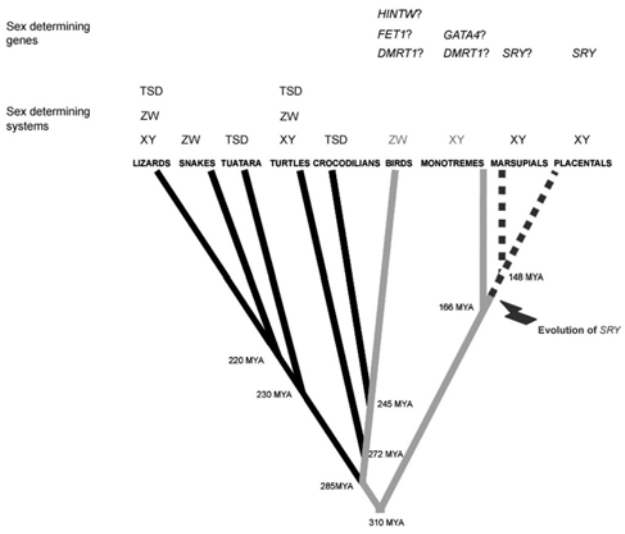


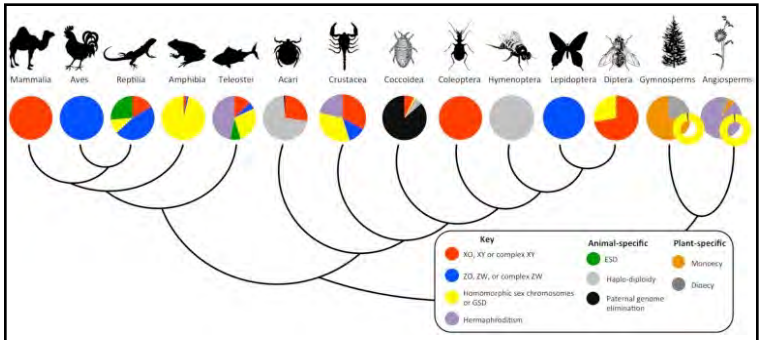


Main genes identified that participate in sex determination in cattle. Blue and orange colors indicate genes participating in the male or female sex determination pathways, respectively. Gray color indicates genes involved in the formation of the bipotential gonad. Crossed genes indicate the main differences found with the mouse model. Underlined genes are those downregulated in males or females on Day 43. Genes crossed out indicate main differences with sex determination in mice.



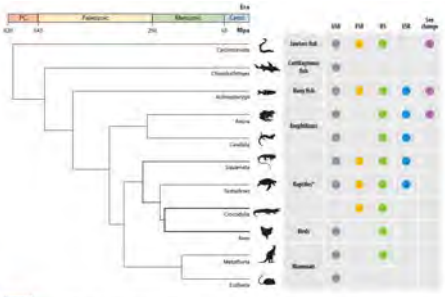
Sex chromosome differentiation.
A. Reconstructed evolutionary path of sex chromosome differentiation in humans. Sex chromosomes originate from autosomes that acquired a sex-determining function (the Sry gene) after their split from monotremes. Suppression of recombination between the sex chromosomes, associated with degeneration of the non-recombining region of the Y chromosome, results in the morphological and genetic differentiation of sex chromosomes. Recombination suppression occurred in multiple episodes along the human X and Y chromosome, forming so-called evolutionary strata. The oldest stratum is shared between eutherian mammals and marsupials, while the youngest stratum of humans is primate-specific. **B.** The degree of sex chromosome differentiation ranges widely across species, spanning the entire spectrum of homomorphic to heteromorphic sex chromosomes, from a single sex-determining locus, as seen in pufferfish, a small differentiated region (strawberry and emu), most of the sex chromosomes apart from short recombining regions (humans), to the entire sex chromosome pair, as seen in *Drosophila*. Note that the sex chromosomes are not drawn to scale.





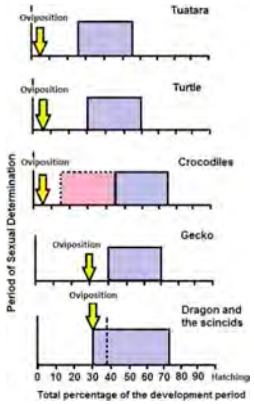
Diversity of sex determination systems for representative plant and animal clades.
The bubble insert graph for the plant clades represents the relative proportion of species with documented sex chromosomes within plants with separate sexes. Vertebrates: Mammalia (placental, marsupial, and monotreme mammals), Aves (birds), Reptilia (turtles, snakes, crocodiles, lizards), Amphibia (frogs, toads, salamanders), and Teleostei (bony fishes). Invertebrates: Acari (mites and ticks), Crustacea (shrimps, barnacles, crabs), and Insects, which include Coccoidea (scale insects), Coleoptera (beetles), Hymenoptera (ants, bees, and wasps), Lepidoptera (butterflies), and Diptera (flies). Plants: Gymnosperms (non-flowering plants) and Angiosperms (flowering plants).

The Genetics and Epigenetics of Sex Change in Fish.
Ortega-Recalde O, Goikoetxea A, Hore TA, Todd EV, Gemmell NJ.
Annu Rev Anim Biosci. 2019 [Epub ahead of print]

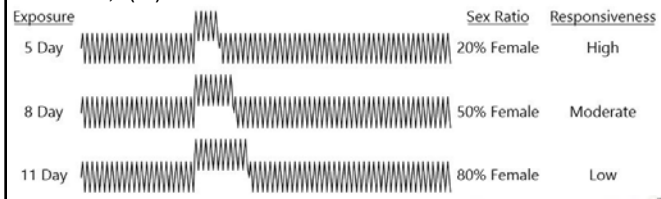


Sex determination and sex change in vertebrates. GSD and ESD coexist in several vertebrate clades for different species. HS during critical time windows affects sex determination in most vertebrate clades except for eutherians, and there is a lack of evidence in chondrichthyans. Fish exhibit remarkable sexual plasticity and may undergo ESR as a result of changes in the external conditions or even complete sex change during adulthood. Reptiles*, nonavian reptiles. Divergence times used to construct the tree were obtained from the TimeTree database (1.14). Abbreviations: Ceno, Cenozoic; ESD, environmental sex determination; ESR, environmental sex reversal; GSD, genetic sex determination; HS, hormone sensitivity; PC, Precambrian.

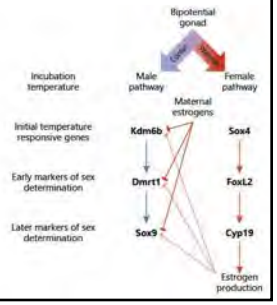
Mechanisms related to sexual determination by temperature in reptiles.
Martínez-Juárez A, Moreno-Mendoza N.
J Therm Biol. 2019 Oct;85:102400.

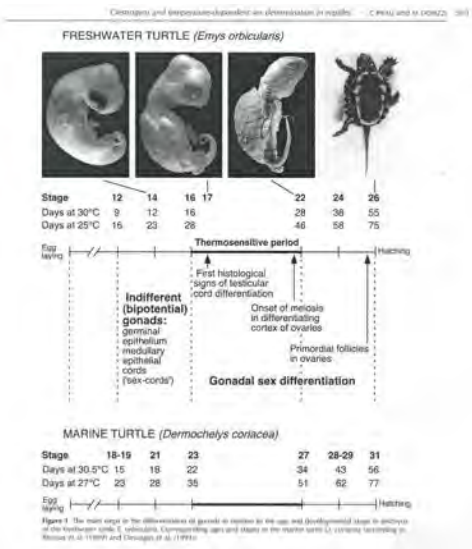


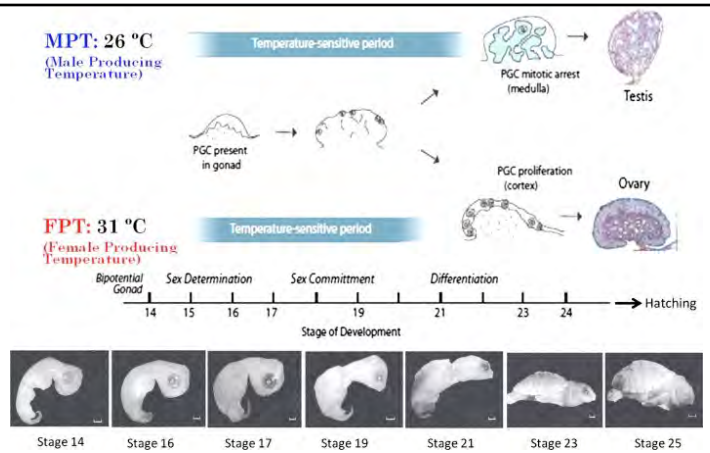
Is Thermal Responsiveness Affected by Maternal Estrogens in Species with Temperature-Dependent Sex Determination?
Bowden RM, Paitz RT.
Sex Dev. 2021;15(1-3):69-79.



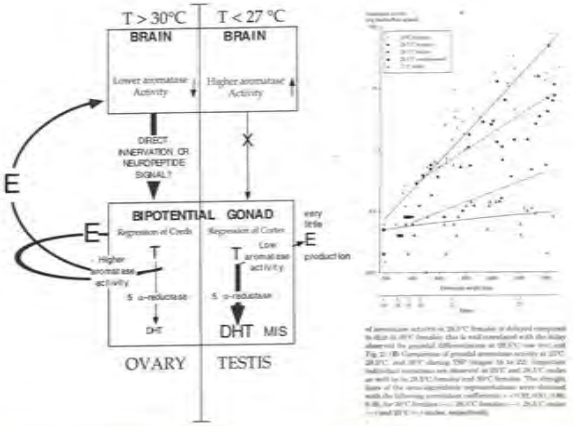
Representative thermal conditions used to characterize variation in thermal responsiveness of T. scripta embryos. Embryos classified as having high responsiveness required only 5 days of exposure to warm temperatures, while those with low responsiveness required 11+ days of exposure to trigger ovarian development. Based on Carter et al. [2018].



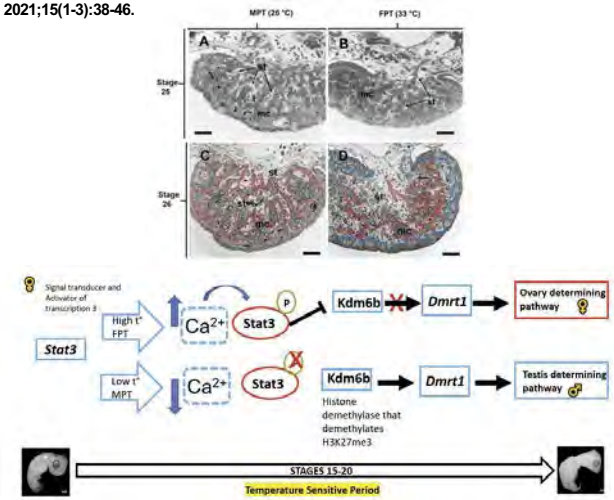




Top: temperature-dependent sex determination and corresponding embryonic developmental stages in the red-eared slider turtle (Trachemys scripta). Bipotential embryonic gonads contain primordial germ cells (PGC) and differentiate to either ovary (at 31 ° C, FPT) or testis (at 26 ° C, MPT) (From Ramsey et al., 2007). Bottom: embryonic development from stage 14 to stage 25 in the red-eared slider turtle (adopted from Greenbaum, 2002).



Molecular and Cellular Mechanisms Underlying Temperature-Dependent Sex Determination in Turtles.
Merchant-Larios H, Díaz-Hernández V, Cortez D.
Sex Dev. 2021;15(1-3):38-46.



Sex Differentiation in Amphibians: Effect of Temperature and Its Influence on Sex Reversal.
Ruiz-García A, Roco AS, Bullejos M.
Sex Dev. 2021;15(1-3):157-167.

Table 1. Effect of temperature on sex ratio in amphibian species

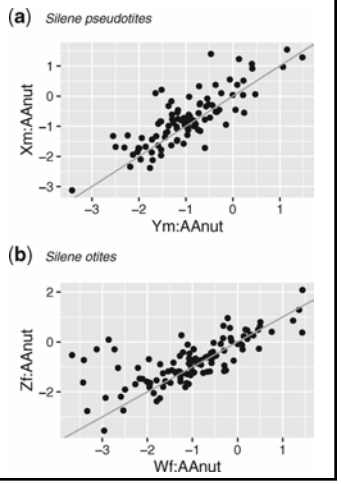
Species	Sex chromosome system	Sex race	Increase temperature	Decrease temperature	Genetic proof of sex reversal
Bufo bufo	ZZ/ZW XX/XY?	Dif	M	F	?
Bufo japonicus	ZZ/ZW?	Dif	M	?	?
Anaxyrus terrestris	?	Dif	Not affected	Not affected	?
Hoplobatrachus rugulosus	?	Dif	M	?	?
Quasipaa spinosa	?	Dif	M	F	?
Euphlyctis cyanophlyctis	?	Semi-dif	M	?	?
Xenopus laevis	ZZ/ZW	Dif	Not affected	Not affected	No
Xenopus polyploid hybrids	WZZ/WZZZ	Dif	Less M	More M	Yes
Rana temporaria	XX/XY	?	M	F (transient?)	?
Pelophylax esculentus (kl. Esculentus)	XX/XY	?	M	M	?
Rana japonica	XX/XY	Dif	M	?	?
Rana chensinensis	?	Semi-dif	M	F	?
Fejervarya limnocharis [Rana limnocharis]	XX/XY	Dif	M	F	?
Rana sylvaticus [Lithobates sylvaticus]	XX/XY	Dif	M	?	?
Rana catesbeianus [Lithobates catesbeianus]	XX/XY	Dif	M	?	?
Hynobius retardatus[a]	?	Dif	F	?	?
Pleurodeles waltl	ZZ/ZW	Dif	M	F	Yes
Pleurodeles poireti	ZZ/ZW	Dif	F	?	Yes
Triturus cristatus	XX/XY	?	M	F	Yes
Triturus carnifex	XX/XY	?	M	F	Yes

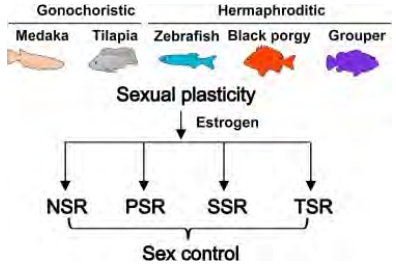
Dif, differentiated; Semi-dif, semi-differentiated. [a] Data from Sakata et al., [2005].

Evolution of Young Sex Chromosomes in Two Dioecious Sister Plant Species with Distinct Sex Determination Systems.
Martin H, Carpentier F, Gallina S, et al.
Genome Biol Evol. 2019 Feb 1;11(2):350-361.

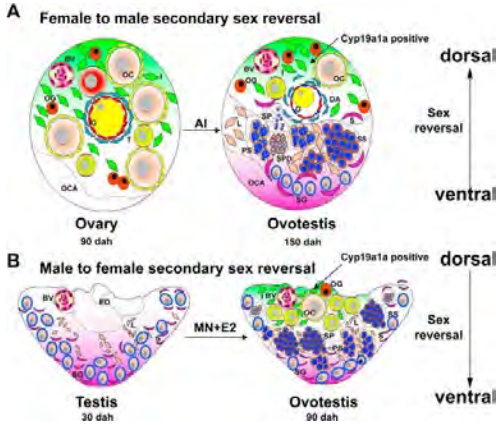
Table 1 Model Comparison Using SEX-DETector on *Silene pseudotites* and *Silene otites*

Species	Sex Determination System	Chromosomal Category	Number of Contigs	BIC
Silene pseudotites	XY	Sex linked	174	3,422,710.589
		Autosomal	7,233	
		Undefined	37,351	
	ZW	Sex linked	0	3,430,155.856
		Autosomal	7,408	
		Undefined	37,150	
	No sex chromosome	Sex linked	0	3,438,114.512
		Autosomal	8,638	
		Undefined	36,120	
Silene otites	XY	Sex linked	222	2,844,016.544
		Autosomal	4,827	
		Undefined	50,005	
	ZW	Sex linked	329	2,644,920.705
		Autosomal	5,232	
		Undefined	49,463	
	No sex chromosome	Sex linked	0	2,862,178.638
		Autosomal	7,008	
		Undefined	48,046	





Sex control in fish by modulating estrogens. Fish maintain their sexual plasticity after sex determination/differentiation in both gonochoristic and hermaphroditic fish. Endogenous estrogens play a critical role in fish sexual plasticity. Sex control in aquaculture can be achieved by modulating estrogens level through artificial primary sex reversal (PSR), secondary sex reversal (SSR), transgenic or knockout sex reversal (TSR).



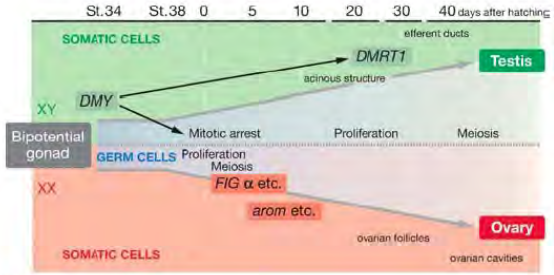
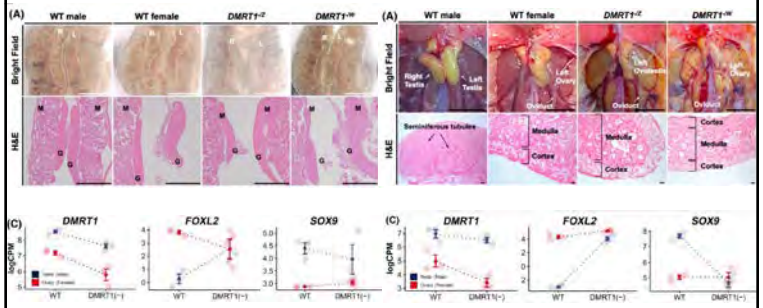


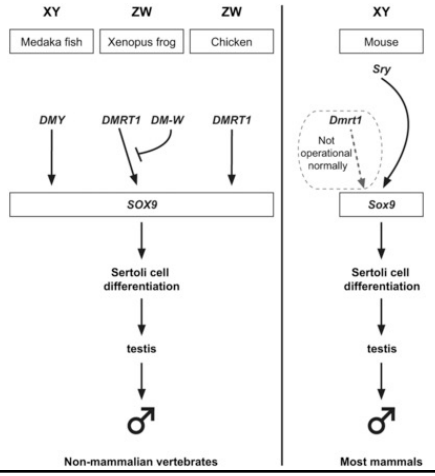
Figure 3

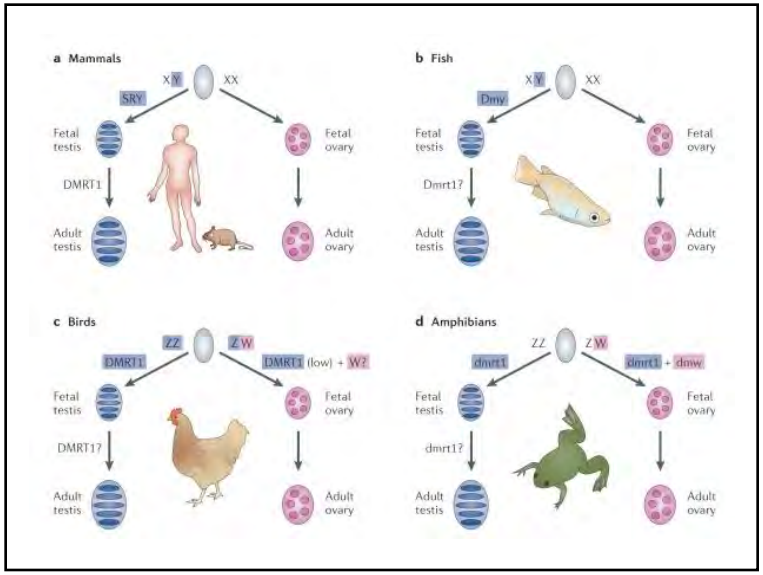
Conceptual illustration of normal sexual development of gonads in the medaka. Morphological events in germ cells are shown in the inner area of gray arrows, whereas those in somatic cells are shown in the outer area of gray arrows. Sex-specific genes expressed in the XY gonad and in the XX gonad are shown in green and pink boxes with black characters, respectively. Black arrows represent putative functions of DMY.

DMRT1 gene disruption alone induces incomplete gonad feminization in chicken
Lee HJ, Seo M, Choi HJ, et al.
FASEB J. 2021 Sep;35(9):e21876.

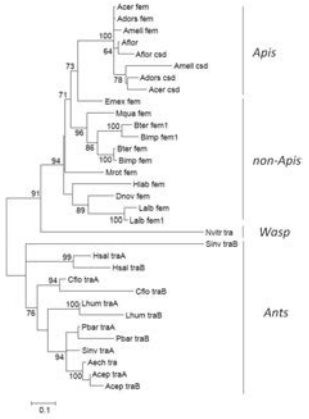


Female-to-male sex reversal in mice caused by transgenic overexpression of Dmrt1. Zhao L, Svingen T, Ng ET, Koopman P.Development. 2015 Mar 15;142(6):1083-8.

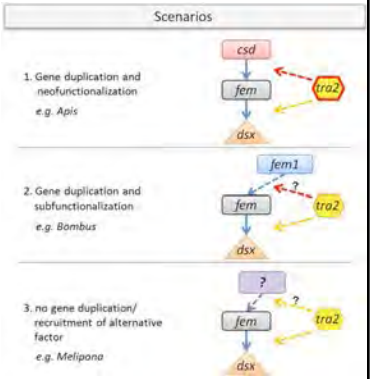




The evolutionary dynamics of major regulators for sexual development among Hymenoptera species. Biewer M, Schlesinger F, Hasselmann M. Front Genet. 2015 Apr 10;6:124



Overview of the evolutionary relationship of the fem gene and copies (fem1, csd, tra) in social insect species.

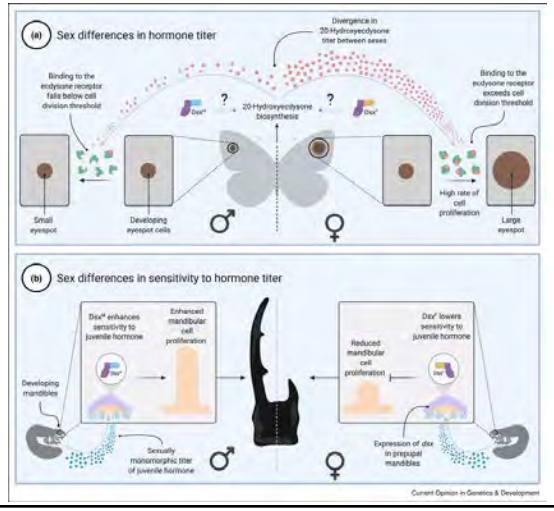


Gene duplication-coevolutionary model for sex determining key regulators in bees.

Table 1. Known master sex-determining genes in vertebrates and insects, and their paralogs.

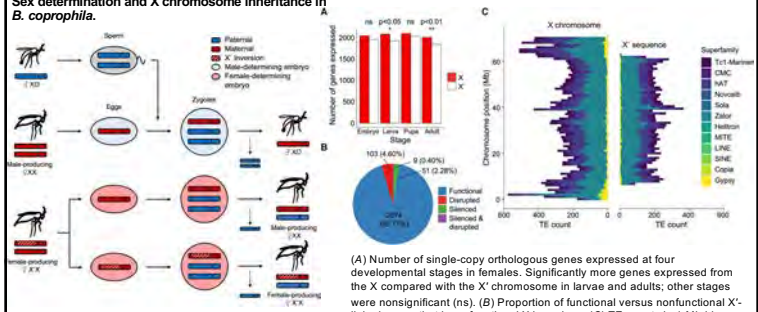
Species	Master sex determining gene	Sex-determining mechanisms	Gene paralog	Paralog function	Reference
mammals	*Sry*	sex-determining Y	*Sox3*	HMG-box transcription factor	[77]
chicken (*Gallus gallus*)	*dmrt1*	dose-dependent Z	-	SD pathway transcription factor	[12]
African clawed frog (*Xenopus laevis*)	*dmW*	sex-determining W	*dmrt1*	SD pathway transcription factor	[13]
medaka (*Oryzias latipes*)	*dmrt1Y*	sex-determining Y	*dmrt1*	SD pathway transcription factor	[78,79]
(*Oryzias luzonensis*)	*gsdfY*	sex-determining Y	*gsdf*	secretory protein in SD pathway	[80]
Patagonian pejerrey (*Odontesthes hatcheri*)	*amhY*	sex-determining Y	*amh*	anti-Mullerian hormone	[155]
rainbow trout (*Oncorhynchus mykiss*)	*sdY*	sex-determining Y	*Irf9*	interferon regulatory factor	[82]
tiger pufferfish (*Takifugu rubripes*)	*amhr2*	dose-dependent X	*amhr*	anti-Mullerian hormone receptor	[156]
smooth tongue sole (*Cynoglossus semilaevis*)	*dmrt1*	dose-dependent Z	-	SD pathway	[14]
fruit flies (*Drosophila*)	*Sxl*	dose-dependent X	*CG3056*	mRNA splicing, non-sex specific	[83,84]
housefly (*Musca domestica*)	*F*	sex-determining W	*tra*	SD pathway switch splice factor	[17]
silkworm (*Bombyx mori*)	*Fem*	sex-determining W	-	piRNA	[85]
honeybee (*Apis mellifera*)	*csd*	haplodiploid	*tra*	SD pathway switch splice factor	[16]
wasp (*Nasonia vitripennis*)	*Nvtra*	haplodiploid	*tra*	SD pathway switch splice factor	[15]

Evolution of sexual development and sexual dimorphism in insects.
Hopkins BR, Kopp A.
Curr Opin Genet Dev. 2021 Aug;69:129-139.



Recent Evolution of a Maternally Acting Sex-Determining Supergene in a Fly with Single-Sex Broods.
Baird RB, Urban JM, Mongue AJ, Jaron KS, Hodson CN, Grewoldt M, Martin SH, Ross L.
Biol Evol. 2023 Jul 5;40(7):msad148.

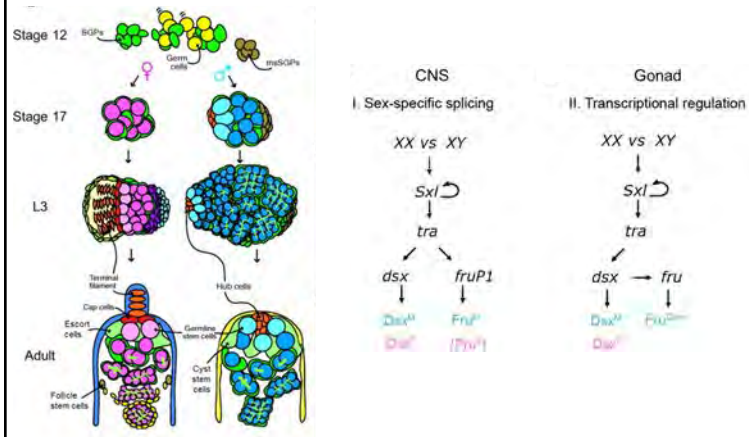
Sex determination and X chromosome inheritance in *B. coprophila*.



(*A*) Number of single-copy orthologous genes expressed at four developmental stages in females. Significantly more genes expressed from the X compared with the X′ chromosome in larvae and adults; other stages were nonsignificant (ns). (*B*) Proportion of functional versus nonfunctional X′-linked genes that have functional X homologs. (*C*) TE counts in 1-Mb bins distributed on the X chromosome and within the X′ supergene sequence.

Our findings provide an indication that sex-linked inversions are driving turnover of the strange sex determination system in this family of flies.

Doublesex regulates fruitless expression to promote sexual dimorphism of the gonad stem cell niche.
Zhou H, Whitworth C, Pozmanter C, Neville MC, Van Doren M.
PLoS Genet. 2021 Mar 31;17(3):e1009468.



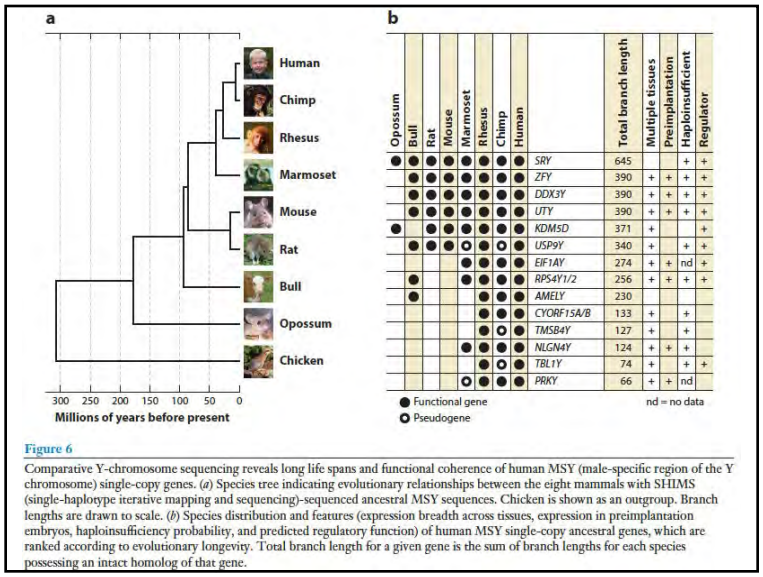
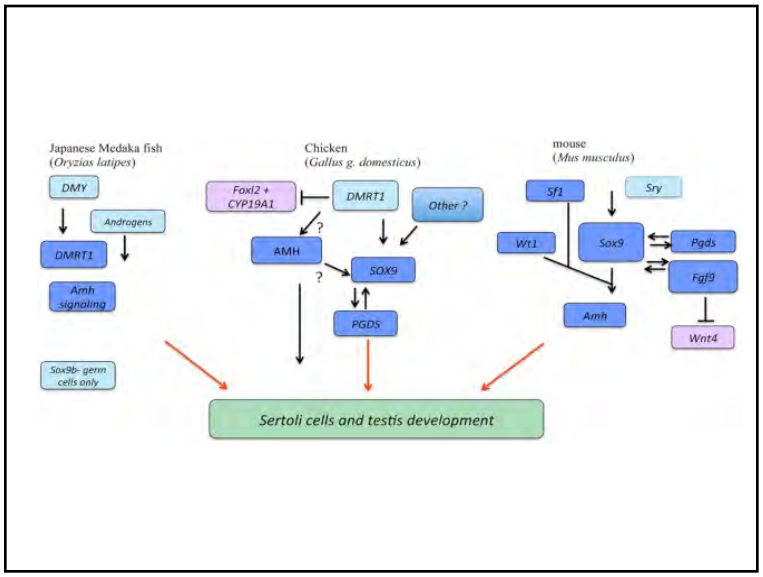
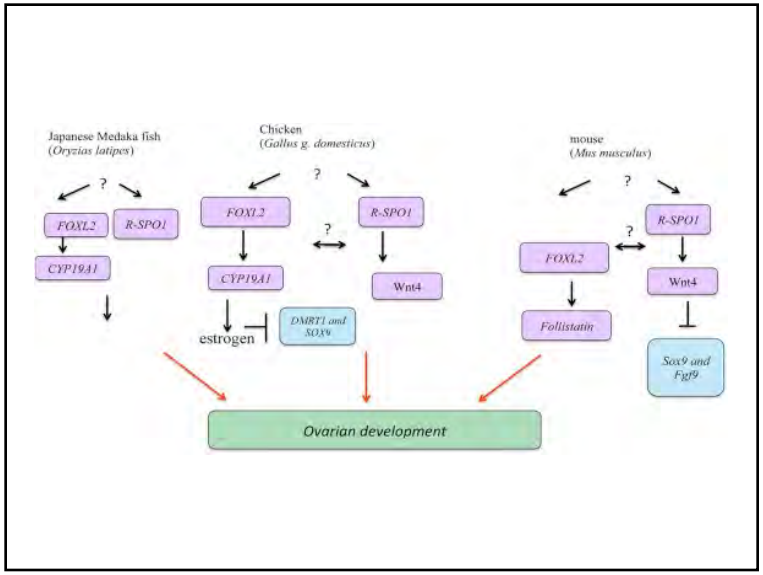
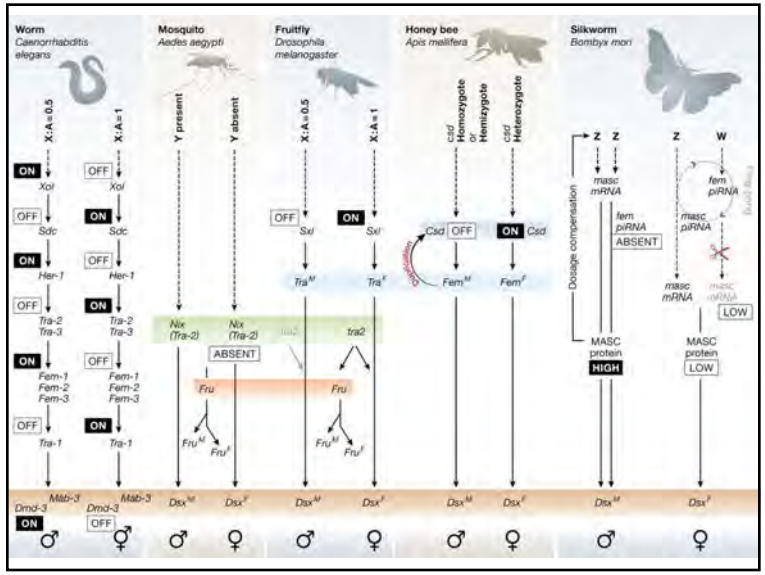


Figure 6

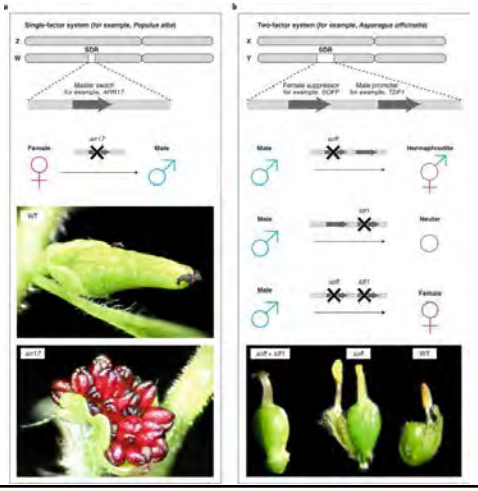
Comparative Y-chromosome sequencing reveals long life spans and functional coherence of human MSY (male-specific region of the Y chromosome) single-copy genes. (*a*) Species tree indicating evolutionary relationships between the eight mammals with SHIMS (single-haplotype iterative mapping and sequencing)-sequenced ancestral MSY sequences. Chicken is shown as an outgroup. Branch lengths are drawn to scale. (*b*) Species distribution and features (expression breadth across tissues, expression in preimplantation embryos, haploinsufficiency probability, and predicted regulatory function) of human MSY single-copy ancestral genes, which are ranked according to evolutionary longevity. Total branch length for a given gene is the sum of branch lengths for each species possessing an intact homolog of that gene.



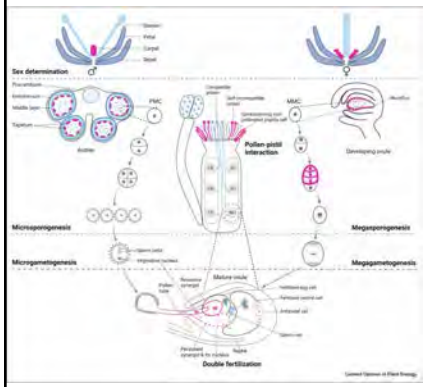




Plant sex chromosomes defy evolutionary models of expanding recombination suppression and genetic degeneration.
Renner SS, Müller NA.
Nat Plants. 2021 Apr;7(4):392-402.



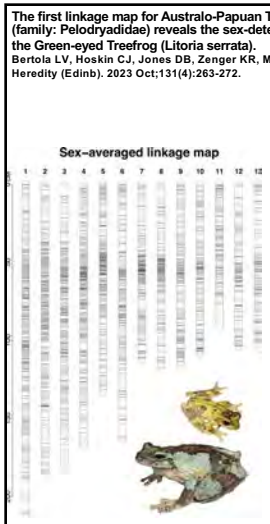
Killing me softly - Programmed cell death in plant reproduction from sporogenesis to fertilization.
Xie F, Vahldick H, Lin Z, Nowack MK.
Curr Opin Plant Biol. 2022 Oct:69:102271.



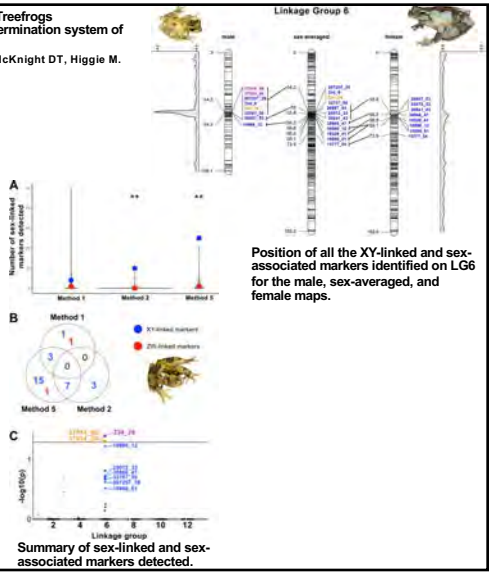
Tissues and cells undergoing developmentally controlled PCD (in magenta) from sporogenesis to fertilization. In unisexual flowers, PCD can cause the abortion or developmental arrest of carpel primordia in male flowers, and stamen abortion in female flowers. During sporogenesis, PCD occurs in supporting sporophytic tissues including the anther tapetum and the nucellus in the developing ovules. After meiosis, non-functional megaspores degenerate. During pollination, some self-incompatibility mechanisms operate by arresting and killing self-pollen on the stigma. Lastingly unpollinated stigmata undergo senescence-triggered PCD, terminating flower fertility. After timely and compatible pollination, the pollen tube grows towards the ovule attracted by the female gametophytic synergids. Upon pollen tube arrival, both the pollen tube cell and the receptive synergid disintegrate, allowing the sperm cells to contact the egg cell and the central cell. The persistent synergid is eliminated after successful fertilization by fusing to the central cell. The antipodal cells in various species do not appear to have specific functions and degenerate before or after fertilization (not covered in this review). Abbreviations: PMC, pollen mother cell; MMC, megaspore mother cell.

Here we shed a light on the latest research into PCD mechanisms in plant reproduction from sex determination over sporogenesis to pollination and fertilization.

The first linkage map for Australo-Papuan Treefrogs (family: Pelodryadidae) reveals the sex-determination system of the Green-eyed Treefrog (Litoria serrata).
Bertola LV, Hoskin CJ, Jones DB, Zenger KR, McKnight DT, Higgie M.
Heredity (Edinb). 2023 Oct;131(4):263-272.



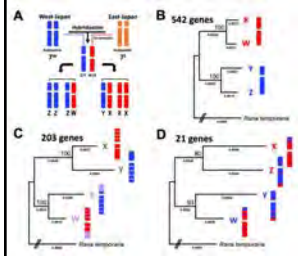
Sex-averaged linkage map for the Green-eyed Treefrog (*Litoria serrata*), showing linkage groups (LG) 1–13.



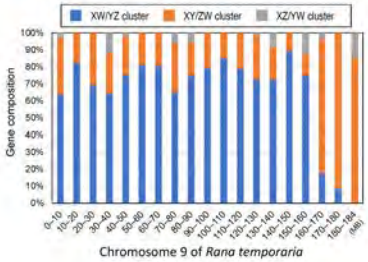
Summary of sex-linked and sex-associated markers detected.

Position of all the XY-linked and sex-associated markers identified on LG6 for the male, sex-averaged, and female maps.

Parallel Evolution of Sex-Linked Genes across XX/XY and ZZ/ZW Sex Chromosome Systems in the Frog Glandirana rugosa.
Mawaribuchi S, Ito M, Ogata M, Yoshimura Y, Miura I.
Genes (Basel). 2023 Jan 18;14(2):257.



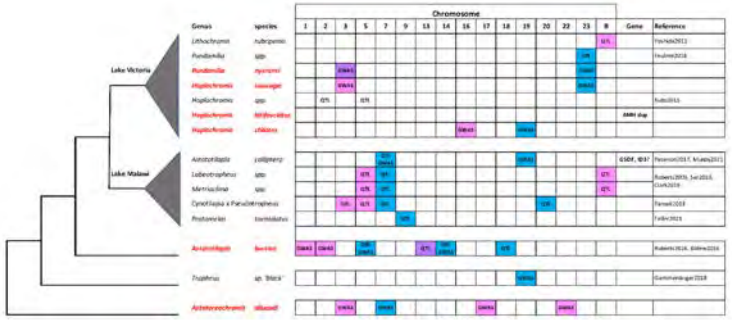
Sex chromosome evolution in Glandirana rugosa and sex-linked gene trees. (**A**) Diagrammatic representation of the sex chromosome evolution in G. rugosa. The XY and ZW sex chromosomes originated from the hybridization between two ancestral type populations of the West-Japan and East-Japan (G. reliquia) groups. X and W chromosomes are indicated in red and the homologous autosome 7 of the East-Japan group (G. reliquia) in orange, while Y, Z, and homologous autosome 7 of the West-Japan group are indicated in blue. (**B**–**D**) Phylogenetic trees were constructed by PAML using 542, 203, and 21 sex chromosome-linked genes (free ratio model) from three different clusters, XW/YZ, XY/ZW, and XZ/YW, respectively. The best-fit models of nucleotide substitution were selected by the Modeltest-NG. Numbers at each node denote the bootstrap percentage values based on 1000 replicates. Numbers below the branches are the expected mean numbers of nucleotide substitutions per site. Rana temporaria was used as an outgroup.



Composition of the sex-linked genes belonging to three different clusters along the chromosomal axis. The genes of the XW/YZ, XY/ZW, and XZ/YW clusters are indicated in blue, orange, and gray, respectively.

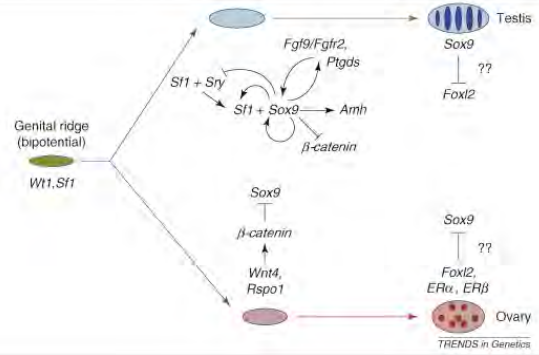
In contrast, the unique genomic region of the sex chromosomes demonstrated a difference between the two systems, with even and extremely high expression ratios of W/Z and Y/X, respectively.

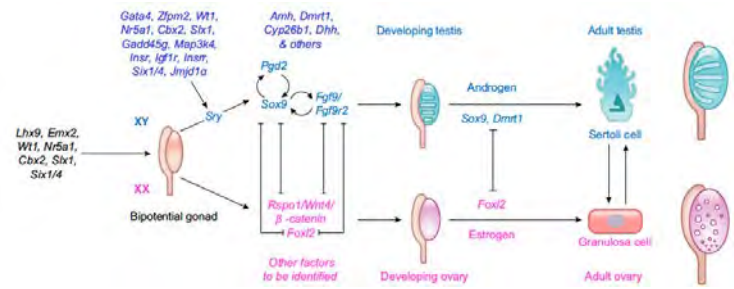
New Sex Chromosomes in Lake Victoria Cichlid Fishes (Cichlidae: Haplochromini).
Kocher TD, Behrens KA, Conte MA, et al.
Genes (Basel). 2022 Apr 30;13(5):804.



Sex chromosomes in the Haplochromini. The phylogenetic relationships of some haplochromine species are shown on the left. The species studied in this paper are listed in red. Blue boxes indicate XY systems, pink boxes indicate ZW systems, and purple boxes indicate instances of XY and ZW variation on the same chromosome in P. nyererei and A. burtoni. Support from QTL or genome wide association studies (GWAS) is indicated, and candidate genes are listed to the right. Yoshida et al., 2011 [13], Feulner et al., 2018 [10], Kudo et al., 2015 [15] Peterson et al., 2017 [53], Munby et al., 2021 [54]; Roberts et al., 2009 [7], Ser et al., 2010 [12], Clark et al., 2019 [14]; Parnell et al., 2013 [16]; Feller et al., 2021 [44]; Roberts et al., 2016 [8], Böhne et al., 2016 [9]; Gammerdinger et al., 2018 [11].

This report brings the number of distinct sex-chromosome systems in haplochromine cichlids to at least 13, and highlights the dynamic evolution of sex determination and sex chromosomes in this young lineage.





"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	**Week 10**	**Spring Break**
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review