Trends in Genetics

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Opinion

Polygenic sex determination in vertebrates – is there any such thing?

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Genetic sex determination (SD) in most vertebrates is controlled by a single master sex gene, which ensures a 1:1 sex ratio. However, more complex systems abound, and several have been ascribed to polygenic SD (PSD), in which many genes at different loci interact to produce the sexual phenotype. Here we examine claims for PSD in vertebrates, finding that most constitute transient states during sex chromosome turnover, or aberrant systems in species hybrids. To avoid confusion about terminology, we propose a consistent nomenclature for genetic SD systems.

Monogenic and polygenic SD

Sex determination (SD) (see Glossary) in multicellular animals initially directs the undifferentiated bipotential embryonal gonad to develop either as testis or ovary. The initiating signal for SD – **genetic SD (GSD)** or **environmental SD (ESD)** – activates a downstream regulatory network that governs male or female gonad development. As a developmental determination process, the downstream network is part of the SD process. However, generally the term 'sex determination' is used to describe the initial, most upstream trigger. The regulation of sexual development after the 'decision' has been made is referred to as **gonadal sex differentiation** [1].

In the best understood GSD systems, SD is inherited as a monogenic trait. Sex chromosomes are defined by a master sex-determining locus that triggers the male or female determining regulatory network. In some systems a male-dominant gene is borne on a male-specific Y chromosome (e.g., *SRY* in mammals [2]) or a female dominant gene on a female-specific W chromosome (e.g., *dmw* in *Xenopus* [3]). Alternatively, a sex-determining gene on the homogametic sex chromosome may be absent or inactive on the heterogametic partner, such that the sex-determining process is one of gene dosage, not dominance. For instance, dosage of the Z-linked *dmrt1* genes determines sex of birds [4] and the Chinese tongue sole [5].

For most species the identity of the SD gene(s) is still unknown, but genetic crosses can usually determine whether one or more independent loci are segregating, and assess the numbers of alleles at these loci. However, genetic crosses cannot exclude the possibility that a whole suite of closely linked genes on a sex chromosome synergistically generate the SD-initiating trigger, so the more conservative terms **unifactorial SD**, **bifactorial SD,** or **multifactorial SD** could be used.

A formal alternative to **monogenic SD** is **polygenic SD (PSD)**, a concept defined by Curt Kosswig [6] (Box 1). The classic definition of PSD is the combined action of alleles of multiple genes at independently inherited loci in one individual to bring about a sexual phenotype. Such a mechanism for SD has since been claimed for various species of plants, insects, fish, and mammals [7–10]. However, major discrepancies in the usage of the term 'polygenic SD' obscure a rich

Highlights

Polygenic sex determination (PSD) is defined as the determination of sexual phenotype by the combined action of two or more genes at independently inherited loci in one individual.

PSD should not be ascribed to multiple alleles at the same sex determination (SD) locus that may be polymorphic in the population, elements of a common biochemical pathway, and to multiple sex chromosomes that are polymorphic in a population, or neo-sex chromosomes or sex chromosome chains when sexual development of the individuum is triggered by one SD locus.

A consistent nomenclature that adheres to accepted definitions of genes and alleles is recommended.

Evolutionary theory predicts that PSD is an unstable state.

Documented cases of PSD are rare. Those examined are best explained by transitionary stages during sex chromosome turnover or aberrant situations in hybrids between species with different sex chromosomes.

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Box 1. A historical perspective on the concept of polygenic sex determination

Sex chromosomes were first described in 1905 [50]. This marked the origin of research on monogenic SD, which has since dominated the common knowledge on how genetic sex determination works. The concept of PSD was broached only about 20 years later and was almost forgotten except by a few specialists. Recently, the field of PSD has been rejuvenated by new results from analyzing the genetics of SD in various species of plants, insects, fish and mammals [7–10].

The term 'polygenic sex determination' was put forward by the German geneticist Curt Kosswig in 1964 [6]. He had already developed the concept in a series of papers since the 1930s. He used the inheritance of sex in hybrids of fish of the genus *Xiphophorus* (platyfishes and swordtails) to postulate a novel genetic mechanism for SD. In his view several male-determining factors (M-factors) and female-determining factors (F-factors) are distributed over the entire genome (see Figure 1 in main text). The individual M and F factors can have different 'strengths' in initiating male or female SD, respectively. He allowed that each M and F factor could exist as different alleles in a population.

Historically, some of the confusion about what PSD is, and what it is not, originated from genetic analyses of a species closely related to those that were used to establish the concept. One year after Kosswig's 1964 paper on PSD, which was built mainly upon sex inheritance in the green swordtail (*Xiphophorus hellerii*, Klaus Kallman detected in another species of the same genus (the southern platyfish, *Xiphophorus maculatus*) the simultaneous presence of X, Y, and W chromosomes in the same population [19]. Kallman clearly recognized that in this species SD is monofactorial and did not use the term 'polygenic'. He even opposed the existence of PSD in platyfish. Since then, Kosswig and Kallman debated whether sex determination in *Xiphophorus* species is polygenic or due to coexisting different sex chromosomes in one species, but they were clear that the two systems represented two different concepts.

Kosswig's usage of the term polyfactorial inheritance was in the sense of phenotypic genetics. He adhered to the definition of a non-Mendelian pattern of inheritance in which a particular trait is produced by the interaction of genes at many loci. The definition of polygenic inheritance that flows from this concept is that a particular trait of an individual is the resultant of the activity of several independent genes at separate loci that act additively. They must work cooperatively in the same individual, rather than merely be polymorphic between individuals in the population.

Classically, this model has been used to describe the inheritance of quantitative traits such as human height or plant leaf size. Each of these polygenes can occur as different alleles in the population so that the quantitative trait loci (QTLs) that interact to produce the phenotype are polymorphic in the population. This results in high variation of phenotypes for a single trait, typically falling into a distribution around a mean. Not all loci contribute equally to the expression of the phenotype; some are considered major QTLs and others minor. In addition, environmental influences can make discerning the polygenic system especially tricky.

One reason thought to account for the rarity of bona fide PSD systems in its strict sense has been the difficulty of detecting polygenes, because loci which contribute only partially and have minor effects on the phenotype are less apparent in analyses of genetic crosses. With the new sequencing technologies, more direct approaches are possible. Methods for identifying sex-specific regions of the genome have been developed from modifications of restriction site-associated DNA (RAD) sequencing [51] and pool-sequencing [52–55]. Such sensitive analyses might bring surprises by detecting minor sex QTLs in systems that have so far appeared to be unifactorial with a major SD locus on a heterogametic chromosome.

Glossary and proposed nomenclature

Bifactorial SD[a]: genetic evidence that the SD mechanism is controlled by two independently segregating loci.

Diallelic SD: a single sex chromosome pair bears a single SD gene, with two alleles present in a population; the inheritance of SD is monogenic.

Digenic SD: two genes at distinct loci act together to activate the male or female sex-determining pathway.

Environmental SD (ESD): the initial trigger that activates the antagonizing gene network comes from the environment (e.g., the temperature during embryonic development in turtles or crocodiles).

Factor: a locus that is inherited as a single unit in genetic crosses. It may consist of one or several genes.

Genetic SD (GSD): the initial trigger that activates the antagonizing gene network comes from the genome of the individual.

Gonadal sex differentiation: the developmental process following sex determination that turns an undifferentiated gonad primordium into either a testis or an ovary.

Monogenic SD: a single gene initiates either the male or the female molecular pathway of SD. The gene acts as a male- or female- dominant, or in a recessive, dosage-sensitive manner.

Multifactorial SD[a]: genetic evidence that the SD mechanism is controlled by more than two separate independently segregating loci.

Polyallelic SD: a single sex chromosome pair bears a single SD gene, with multiple alleles present in a population; as opposed to monoallelic SD, where only one allele is present in a population. The inheritance of SD is monogenic.

Polygenic SD: sexual phenotype determined by the combined action of alleles of multiple genes at independently inherited loci in one individual.

Sex determination (SD): the developmental process that establishes whether the bipotential gonad primordium will become a testis or an ovary. On the molecular level it tilts the balance in favor of pro-male or pro-female developmental processes in mutually antagonizing gene networks.

Unifactorial SD[a]: genetic evidence that the SD mechanism is controlled by a single locus.

[a]: We use Greek prefixes for 'genes' and 'alleles' (words with Greek roots), but Latin prefixes for 'factors' (words with Latin roots).

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diversity of distinctly different mechanisms of multifactorial SD. We critically examine vertebrate systems described as having PSD and offer a consistent nomenclature (see Glossary).

Theoretical considerations

SD is unique in that it channels a single embryonic tissue into one of two alternative fates. This is very different from quantitative trait loci (QTL) that vary continuously, in which many independent genes combine to deliver the phenotype, and values are distributed around a mean. Quantitative characters are typically determined in a polygenic fashion.

So, first, how could a quantitative genetic signal in PSD produce a dichotomous qualitative output? Kosswig proposed that the sexual phenotype is determined by the additive effects of many male (M) and/or female (F) **factors** to reach a threshold value [6]. If the sum of 'activity' of M factors is higher than that of the F factors, male development would be initiated, and *vice versa* (Figure 1). At the molecular level, gene activity is controlled in many ways, making gene expression usually quantitative. If a transcript threshold level is required to produce a phenotype, the output will be qualitative. Indeed, this must be the case for temperature-dependent SD (TSD), whereby a
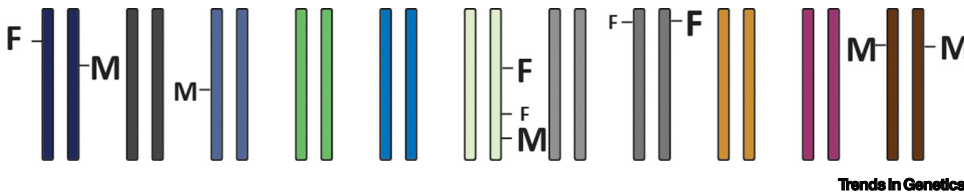
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Figure 1. Kosswig's model of polygenic sex determination. F, female determining factors; M, male determining factors; the size of the letter indicates male- or female-determining strength. If the sum of M > F, male development is initiated; if M < F, females will develop.

continuous variable (temperature of egg incubation) generates a qualitative signal to direct either male or female development of the embryo.

Second, how stable would a PSD system be? Much theoretical work suggests that PSD is evolutionarily unstable in the long run [11,12] but may be a transient state during turnover from one monogenic system to another [7]. However, there are models to explain how sexually antagonistic alleles at multiple loci could maintain a stable polymorphism [9].

One consequence of polygenic inheritance would be a wide variation of sex ratio among families, depending on where the threshold was set [12]. Sex ratio parity could be achieved only by coincident evolution of allelic frequencies for the multiple sex genes and a strict threshold for the production of one sex over the other. However, if one sex is produced in excess, individuals that produce higher proportions of the rarer sex among their offspring have the advantage of leaving more grandchildren (under Fisher's frequency-dependent selection). Thus, alleles at the PSD loci that favor the production of the minority sex will spread, but their selective advantage will diminish progressively until a 1:1 sex ratio is achieved. This inherently unstable system evolves to a 1:1 ratio as a single sex-determining gene takes over.

Overall, evolutionary pressure on PSD systems would be expected to enhance the binary nature of gonad development, because individuals with any kind of intermediate phenotype are likely to be infertile, and therefore the alleles they carry are heavily selected against. We might therefore expect monogenic systems of SD to be most common in vertebrates. As we show later, this is indeed what we observe.

Fish with multiple SD genes

Much of the evidence for PSD comes from studies of SD in a few fish species. A two-factor system was described for the swordtails *Xiphophorus multilineatus* and *X. nigrensis.* They have a basic XX/XY SD system, but occasionally XX males result from homozygosity of alleles of a sex-modifying autosomal locus. In AA fish, XX genotypes develop normally as females, but aa determines male development of XX fish. Thus gene A/a is considered an autosomal modifier of a sex chromosomal system [13].

In African cichlid fish (e.g., *Metriaclima pyrsonotus* [14]), alleles at an XY locus and a WZ locus in two different linkage groups segregate independently. The presence of the W overrides the male-determining effect of the Y, so ZWXY fish develop as females, constituting a bifactorial system. However, in a related species, *M. mbenjii*, ZWXY females have intersex-like phenotypes for some traits, and female phenotypes for others [15], suggesting that this system is not at evolutionary equilibrium.

The European sea bass (*Dicentrarchus labrax*) has been described as having PSD, with more than three QTLs associated with sex ratio [16]. The temperature to which young fish are exposed

interacts with genomic determinants to promote either male or female gonad development. Intra-testicular oocytes are frequent in young sea bass males [17], and there are population-specific variations of the genetic components of SD [18], again suggesting a species in transition.

Thus, in fish there are several sex-determining systems controlled by more than a single factor. However, it is unclear which represent stable polygenic systems and which transient states, a question that we examine further.

Multiple variants at the same locus do not qualify as 'polygenes'

Other cases of multiple sex chromosomes, however, do not fit the definition of PSD.

In the Southern platyfish (*Xiphophorus maculatus*), X, Y, and W chromosomes are simultaneously present in the same population [19]. The W overrides the male-determining activity of the Y, so males can be either XY or YY and females XX, WX, or WY. But the three sex chromosomes clearly belong to the same linkage group. Hence, we refer to this system as monofactorial (Box 1). Whether the SD loci on the three chromosomes harbor different alleles of the same gene or different genes is unknown.

Similarly, the wrinkled frog (*Glandirana rugosa*[20]) has XX/XY and ZW/ZZ populations on two different Japanese islands, with a hybrid zone on a third island, where various combinations of these four sex chromosomes occur. Gene mapping shows that XY and two different WZ systems are variants of the same chromosome [21], and that the most likely SD gene *SOX3* is shared [22]. Thus, we would classify this SD system as monogenic and **polyallelic**.

Changes in SD gene activity may be brought about by mutation at the SD locus itself, or at another locus on the sex chromosomes or an autosome. This is the case in several rodents in which *Sry* is inactivated or inhibited. In six species of Patagonian akodont mice, a polymorphism for an unknown change on a variant Y* [23] produces XY* females [24]. No sequence differences in *Sry* were observed between XY males and XY* sex-reversed females, so this may constitute a system of SD control by two separate but linked Y-borne factors (digenic SD). Alternatively (and perhaps more likely), *Sry* transcription from Y* may have been expunged by mutation in the promotor or upstream regulators that were not sequenced, which would constitute a system that is monogenic and polyallelic.

In several rodents – including the wood lemming and the African pygmy mouse – a variant X chromosome (X*), polymorphic in the population, inhibits the male-determining effect of the Y, so that X*Y animals develop as females [24,25]. The molecular identity of the X-linked locus that suppresses the action of *Sry* is unknown. If another locus on the X evolved to interact with SRY, we would describe the system formally as 'digenic'. However, if X* has an allele of *Sry* or its X-borne homolog *Sox3* that overrides the wild-type *Sry* action, this SD system would be monogenic and **diallelic** (polymorphic for X variants).

Thus, identifying the modifier locus, or at least documenting its independent assortment, is essential in classifying these SD systems.

Multiple genes in a biochemical pathway do not qualify as 'polygenes'

Known SD master genes all enact SD through a complex biochemical pathway. The Y-borne mammalian *SRY* turns on *SOX9*, which in turn activates genes such as *AMH* and *DMRT1* in the testis-determining pathway. In the absence of *SRY*, other genes such as *WNT4* and *FOXL2* are activated that promote the development of an ovary. Testis- and ovary-promoting pathways

interact through cross-suppression, so that the outcome depends on a delicate balance that is easily upset by a mutation of any of the components, resulting in various degrees of sex reversal. In birds, dosage effects of the Z-borne SD master gene *DMRT1* directs promotion of testis or ovary via alternative pathways that share many genes with mammals. So even regulation of sexual differentiation directed by monogenic SD depends on multiple (at least 60) genes [26,27].

However, we would not consider that these genes are acting as polygenes, since they are all under the control of a single master sex-determining trigger. The downstream genes do not deliver the primary signal for SD, but are the receivers of the signal.

Multiple sex chromosomes do not contain 'polygenes'

In some animals (mammals, frogs, and fish) as well as some plants, sex chromosomes are fused or translocated with autosomes to produce multiple sex chromosomes that segregate as meiotic chains. The male platypus has five Y and five X chromosomes, which form a meiotic chain that segregates alternately. The five X chromosomes go to one pole and the five Y chromosomes to the other, producing only two types of sperm that produce male and female offspring. Thus, they behave as a single X and Y chromosome [28] and SD is unifactorial. Identification of *AMH* (anti-Mullerian hormone) as a candidate master SD gene on Y_5 [29,30] is consistent with monogenic SD.

Similarly, the Brazilian fish piapara (*Megaleporinus elongatus*), has a $Z_1Z_1Z_2Z_2$ male/$Z_1W_1Z_2W_2$ female type of SD [31]. Z_1–Z_2 as well as W_1–W_2 segregate together, again signifying unifactorial (assumed monogenic) SD.

In several species, one of the sex chromosomes is fused with an autosome, generating composite neo-X, neo-Y, or neo-W chromosomes. There has been speculation on whether the added bits contain sex-determining loci that must be inherited together as polygenes, but there is no evidence to support this attractive hypothesis. Indeed, comparative gene mapping shows that a large autosomal region was added to both the X and the Y of eutherian mammals 105 million years ago [32] and is the origin of most human Y chromosome genes [33]. However, the 23 genes in the Y added region play no role in human SD, which is entirely controlled by *SRY*.

What if the chromosome complement doubles, as occurred twice in the common ancestor of all vertebrates, and again in several lineages of fish and amphibians? XY species would therefore have four sex chromosomes, and an SD gene on the Y would be present in two copies, qualifying as polygenes that segregate independently. Various combinations of gametes would generate ¼ XXXX, ½ XXXY and ¼ XXYY embryos, distorting the sex ratio or creating infertile intermediates, depending on effects of Y dosage [34]. We know of no polyploid vertebrates with duplicate sex chromosomes. Rather, the tetraploid frog *Xenopus laevis* solved this problem by inventing a novel ZW [35] that bears a duplicated and truncated version of *DMRT1* to craft a new female-dominant W that inhibits male development [3].

Thus, multiple and composite sex chromosomes generated by translocation or polyploidy do not qualify as systems of PSD.

Species hybrids

Species hybrids may have problems with incompatible sex chromosomes. Indeed, interspecies crosses often produce aberrant sex ratios [14,36]. This incompatibility is often resolved by the evolution of various parthenogenic ('unisexual') hybrid species amongst fishes, amphibians, and reptiles [37], which reproduce as all-female biotypes.

However, species hybrids may reveal many genes with major and minor effects on SD. In a recent study [38] two species of catfish were crossed, and genotyping of multiple SNPs was used to detect seven male-associated and 17 female-associated loci that map to different linkage groups (so are not allelic). This abnormal situation may, indeed, be polygenic.

Evolutionary replacement of an SD system

The best-known chromosomal SD systems (in mammals, birds, and fruitflies) are extremely stable. However, reptiles, amphibians, and fish show tremendous SD variety. Several different SD genes have been identified, often in closely related species, indicating that SD systems can frequently turn over as one monogenic system is replaced by another.

How can a new SD system take over from the old? If the emerging novel sex chromosomes evolve from a different autosome, we might expect odd mixtures of sex chromosomes, which would be disadvantageous. For instance, if a new ZW system replaced an old XY system, XXZZ individuals or XYZW might be intersex or at least infertile. Alternatively, one system might prove to be epistatic to the other. This well describes the two-factor (bifactorial) system of the African cichlid, in which alleles at an XY locus and a WZ locus on two different chromosomes segregate independently and the W factor is epistatic to the Y, suggesting that it is, indeed, a species in transition.

Modeling shows that ZW–XY transitions may evolve with changes in the threshold for the decision on male or female fate [39] without substantive genotypic innovation. The master sex gene and sex chromosome pair can be retained, the Z becoming the Y and the W becoming the X. If a nascent sex chromosome evolves from the same linkage group (for instance a W evolves from an X in an XY system and is epistatic over Y), the three sex chromosomes can coexist until the Z emerges, possibly from the now functionless Y. The unifactorial WXY systems in platyfish and the wrinkled frog may represent such a scenario.

It is interesting to consider the evolution of SD modifiers as extensions to the sex-determining pathway as a first step in sex change, and to consider whether this situation leads to the evolution of a new monogenic system. For instance, the A/a locus of *Xiphophorus*, and the X* repressor of Sry in lemmings, may constitute such extensions of the pathway, in accordance with Wilkin's 'bottom up' hypothesis [40,41].

There are several situations (e.g., in the frog *Rana temporaria* [42]) in which a new XY or WZ system can be detected in a population as a cline of frequencies with which different linkage groups determine sex.

Captive zebrafish colonies are particularly interesting. Many studies in independently maintained colonies gave inconsistent results for sex linkage to one of three different chromosomes; sex ratios were far from 1:1, and the environment was thought to play some role in this. However, studies of wild zebrafish show an unequivocal ZW system [43]. It is proposed [44] that female-to-male sex reversal of fish with the ZW genotype during repeated rounds of gynogenesis and the process of domestication led to the eventual loss of the Z chromosome. The domesticated zebrafish strains are composed of only WW genotypes, some of which become females and others become fertile neomales. Different colonies have been independently evolving novel SD systems for only about 30 years. The sex ratios and inconsistent sex linkage suggests that downstream genes in testis and ovary pathways are battling it out for supremacy: a situation about as close to 'polygenic' as we might get, and a wonderful opportunity to study the first steps in the evolution of new SD genes. A recent study indeed provided evidence that a domesticated zebrafish stock turned to PSD after the loss of the monogenic system [45].

In the Siamese fighting fish breeds that are the result of strong artificial selection and hybridizations, some strains are monogenic for *dmrt1* as a male determiner [44] while others show polygenic sex inheritance that may denote the invasion by a novel sex-determining region [46].

A loss of the W chromosome has also been documented for the Australian dragon lizard *Pogona vitticeps*, whose ZZ male/ZW female system can be overridden at high temperatures, producing ZZ females. Mating sex-reversed ZZ females to normal ZZ males produces all ZZ progeny whose sex is completely determined by incubation temperature [47]. Likewise, in the medakafish, frequent loss of the Y chromosome that bears the master male SD gene (a duplicated copy of the autosomal *dmrt1a* gene), was observed in laboratory populations [48]. Under environmental stress (elevated temperature), and mediated by cortisone, the autosomal *dmrt1a* became precociously activated in embryos and mimicked the action of the Y-chromosomal *dmrt1bY* [49]. As a result, XX fish developed as males with a novel proto-Y chromosome, which eventually supplanted XY males.

Concluding remarks

Where we find vertebrate systems with multiple sex-determining factors, we must ask (i) whether there are multiple independently inherited genes acting in the same individual, with combined effects on sexual phenotype, rather than constituting polyallelic systems or several steps in a biochemical pathway, (ii) whether these genes act in the same individual or are merely polymorphic in the population, and (iii) whether this is a stable relationship or a stage in turnover when one system is taking over from another. On this definition, most instances of multiple SD genes fail to qualify as PSD (Figure 2).

Indeed, the only examples of *bona fide* PSD that we have been able to identify in vertebrates can be considered to be very young, transitioning, or very disturbed SD systems. The digenic systems and intersex phenotypes seen in some fish may represent intermediates in turnover, and the captive zebrafish populations that accidentally lost their Z chromosome over the past few decades [43] represent an extremely early stage. The catfish species hybrids [38] represent a deranged system in which the SD genes of the two systems are fighting it out.
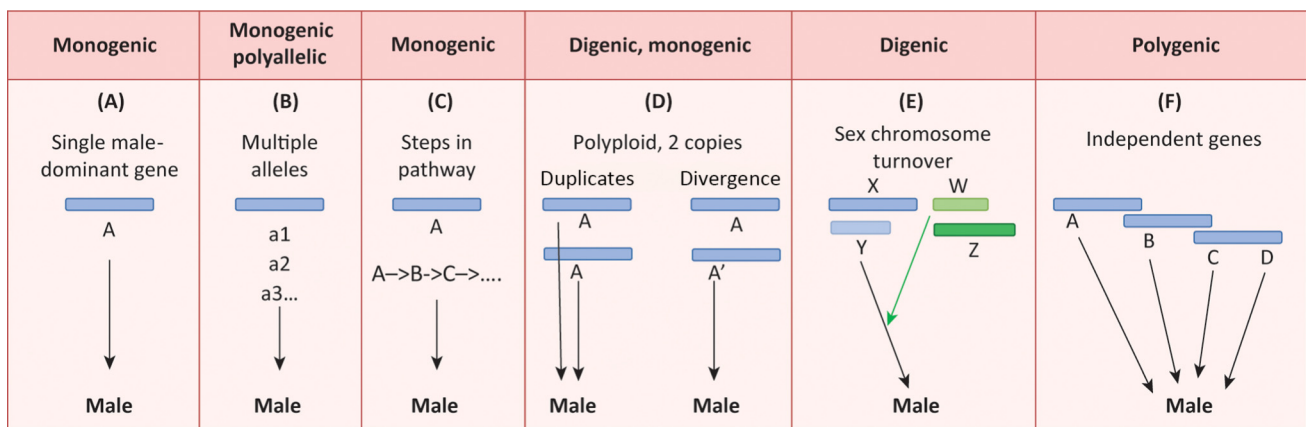
Figure 2. Monogenic, digenic, and polygenic male determination. The first three panels denote monogenic sex determination, since a single gene A on a sex chromosome (blue bar) directs male determination, either by (A) its presence/absence, (B) through multiple alleles, or (C) by initiating a biochemical pathway. Panel (D) represents a tetraploid with two copies of gene A on two copies of the sex chromosome; this would produce a digenic system if both A copies were active (e.g., via A dosage), or would revert to a monogenic system if one copy A' degenerates or changes its function. Panel (E) illustrates a digenic system in transition in which an original XY system is being taken over by a novel WZ system. Panel (F) illustrates a truly polygenic system in which four genes at distinct loci on three chromosomes contribute additively toward male determination. Blue bars, male-promoting chromosome systems; green bars, female-promoting chromosomes.

To broaden our knowledge about the occurrence of PSD and to enhance our understanding of the evolutionary forces that lead to this system and eventually maintain it (see Outstanding questions) we need more information about the genetics and molecular developmental biology of sex in many more species. Our picture is far from complete.

We conclude that, as predicted by evolutionary arguments, monogenic systems of SD predominate in vertebrates. Truly polygenic systems are rare, and represent transitions between systems, or deranged systems in species hybrids.

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