Trends in Genetics

CellPress

Opinion

Yes, polygenic sex determination is a thing!

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The process of sexual development in animals is modulated by a variety of mechanisms. Some species respond to environmental cues, while, in others, sex determination is thought to be controlled by a single 'master regulator' gene. However, many animals respond to a combination of environmental cues (e.g., temperature) and genetic factors (e.g., sex chromosomes). Even among species in which genetic factors predominate, there is a continuum between monofactorial and polygenic systems. The perception that polygenic systems are rare may result from experiments that lack the statistical power to detect multiple loci. Intellectual biases against the existence of polygenic sex determination (PSD) may further arise from misconceptions about the regulation of developmental processes and a misreading of theoretical results on the stability of polygenic systems of sex determination.

Highlights

In many species, the process of sex determination responds to a combination of environmental and genetic factors.

'Master sex determiners' is an outdated and inaccurate description of the structure of developmental regulation.

Additive effects of several genes do not produce unfit intersex individuals, because continuous genetic variation is channeled into discrete phenotypes via thresholds or switches in developmental processes.

Polygenic sex determination (PSD) has been identified in numerous species and can persist for long evolutionary periods.

The true extent of PSD has likely been underestimated due to bias in the experimental methods that have been used to identify genes affecting sexual development.

Population genetic theory shows that systems of PSD can be evolutionarily stable.

Genetic basis of sex determination

A fundamental phenotypic distinction among individuals in animal populations is whether they produce sperm (males), eggs (females), or both (sequential or simultaneous hermaphrodites). Individual embryos typically have the potential to develop as either sex. The switch between male or female development can be initiated by genetic differences, environmental factors, such as temperature, or possibly by random developmental noise [1]. The relatively discrete nature of the sexual phenotype, and its correlations with patterns of chromosome segregation [2], contributed to the view that sexual development is controlled by single Mendelian factors. An alternative view is that sexual development is regulated by two or more independently segregating genes, that is, is polygenic [3]. The latter view was supported by studies of *Drosophila*, in which sex is not determined by the presence or absence of the Y chromosome, but rather by the dosage of several X chromosome-encoded proteins [4].

Nevertheless, the paradigm that sex is genetically controlled by variants of a single genetic locus, referred to as a '**master sex determiner**' (see Anti-Glossary) or 'master key regulator' of gonad development [5], remains dominant. These genetic variants are supposed to act as 'triggers', which control 'slave' genes to direct development into male or female **pathways** [6,7]. This perspective gives prime agency to the gene, overlooking the totality of the complex regulatory process in which the DNA is just one player.

The chromosome pairs that carry such key regulatory variants are called sex chromosomes. From a Mendelian perspective, some species have a dominant male-determining allele on a Y chromosome (male heterogametic XX–XY systems), while other species have a dominant female-determining allele on a W chromosome (female heterogametic WZ–ZZ systems) [8]. The molecular mechanisms of dominance are diverse and include both gain- and loss-of-function mutations [9]. The molecular difference between these alleles can be as small as a single nucleotide substitution, but a variety of evolutionary forces contribute to the accumulation of large numbers of sequence differences between the X and Y (or Z and W) chromosomes [10], until, in

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some cases, they can be distinguished under a light microscope. Given that the Y chromosome is always found in males, selection favors the accumulation of alleles on the Y that increase the fitness of males [11]. This form of sexually antagonistic selection occurs on each of the sex chromosomes. Selection may also favor mutations that reduce recombination between the sex locus and the sexually selected alleles, leading to large regions of linkage disequilibrium adjacent to the sex locus. Often this selection leads to fixation of structural variants (e.g., inversions) around the sex locus that drastically reduce recombination between the sex chromosomes.

In a recent opinion in *TiGS* [12], Schartl *et al.* argued that a single gene (a master sex determiner) controls **sex determination** in nearly all vertebrate species. They claim that PSD is rare and typically a transient phenomenon during the evolutionary replacement of a sex-determining gene, or an aberration found in interspecific hybrids. They also propose definitions to exclude many established examples of PSD.

Here, we discuss some misconceptions about how information from polygenic systems is interpreted by developmental processes to produce discrete sexual phenotypes. We assemble the evidence for stable systems of PSD and argue that its prevalence has been underestimated. Finally, we review population genetic theory that identifies several different selective forces that can maintain evolutionarily stable systems of PSD.

A continuum between GSD and ESD

It has been common to make a distinction between genetic and environmental systems of sex determination [13]. Most mammals are considered to have a purely genetic system based on a Y chromosome containing the SRY gene, while many reptiles are considered to have systems of environmental sex determination (ESD) based on nest temperature [14]. However, both genetic and environmental contributions affect the probabilities of sexual development. The bearded dragon (*Pogona vitticeps*) has a female heterogametic (ZW) system of genetic sex determination (GSD), but ZZ males may be sex-reversed to female by high temperatures [15,16]. There are essentially infinite possibilities for systems with different contributions of genes and environmental variables. Therefore, sex determination is a continuum [17,18], in which the quantitative contributions of genes and environment are canalized by the developmental process.

Individual species fall at various points along this continuum, with some being primarily GSD and others primarily ESD. The Atlantic silverside (*Menidia menidia*) shows variation in temperature sensitivity along a latitudinal gradient [19]. In southern populations, sex is largely determined by environmental factors, with low temperatures promoting female development. However, there is considerable variation in response among families, suggesting a polygenic basis to temperature sensitivity. In northern populations, sex is largely determined by genetic factors, with little or no response to differences in rearing temperature [20]. The European sea bass (*Dicentrarchus labrax*) has a PSD system subject to strong environmental influence [21]. High temperatures during early development (<100 days) promote male development by inhibiting female-expressed genes and upregulating male-expressed genes. Conversely, low temperatures over longer periods promote male development by significantly reducing growth and blocking normal ovarian differentiation [22]. Sex determination in tilapia (*Oreochromis* spp.) is largely influenced by a few genes with major effects, but wild populations frequently experience high temperatures, which can masculinize the larvae. Thermosensitivity varies among populations and is influenced by genetic factors [23,24]. The mixed GSD/ESD systems in these species are not transient states, but are instead stable mechanisms shaped by selective pressures unique to their particular ecologies. Genetic variation in environmental sensitivity among families is prima

Anti-Glossary

A short dictionary of misunderstood words commonly used in the sex determination literature. These terms should be carefully defined in context, and some should be completely avoided.

Pathway: there is no such thing as a pathway, biochemical or developmental, responsible for sex determination. Rather, linear pathways are simply portions of heritable homeostatic regulatory networks that have been removed from their context. Homeostatic systems are constructed of feedback loops, not linear chains.

Sex determination: this phrase can mean different things depending on the context, much like the word 'gene'. We argue for greater precision in its use. First, it is inappropriate to talk about sex determination as a single event in the development of an organism. In many species, male or female sex is a state that must be actively maintained throughout life [137]. In sequential hermaphrodites (e.g., sex-changing fishes), sex is not 'determined' by genes, but instead represents two stable regulatory states of an individual. Second, rather than erect distinct categories of genetic or environmental sex determination, we should identify testis- or ovary-promoting factors (or somatic equivalents) and focus on the stability of the homeostatic regulatory systems that maintain a particular sexual state. Third, developmental processes often differ among tissues; thus, we should take care to distinguish, for example, somatic versus gonadal sex determination, because different mechanisms may be involved.

(Master) sex determiner: the idea that a single gene determines sex is wrong. This term dates from the early days of Mendelian genetics, when it was noticed that variants at a particular locus had a major effect on the phenotype. However, no gene acts in isolation. It can only exert its effect on the phenotype within the context of a particular regulatory system/environment. The idea of a 'master' regulator of development was introduced by Ohno [5], who believed that the expression of the many thousands of mammalian genes must be organized by a regulatory hierarchy controlled by a small number of genes. However, this was a hypothesis, not a discovery.

'Top-level' sex determiner: if developmental systems are not linear

facie evidence for PSD because it indicates heritable differences in sexual development at a given temperature [25].

Regulatory architecture of developmental systems

We next consider how the genetic factors influencing sexual development act within developmental networks. The discovery of the structure of DNA was coincident with the development of digital computers, giving rise to analogies of the genome as an architectural blueprint, or set of instructions for the construction of organisms. This perspective currently dominates biological thought, with much effort going toward the elucidation of gene-based regulatory networks. However, DNA does not have agency, but rather functions only within a metabolic process that includes the cellular environment of RNA, proteins, and other molecules that determine which genes are expressed. Our focus on genes as things, rather than as components of a process, has distorted our understanding of the heritable homeostatic regulatory architectures that underlie development [26]. Organisms exist through the perpetuation of heritable (homeostatic) regulatory architectures, and not simply by information flowing from DNA, which is an inert molecular incapable of creating a phenotype on its own.

'Master sex determiner' is misleading

The idea that a single genetic factor controls sex determination can be traced to the early days of Mendelian genetics and the cytological identification of segregating sex chromosomes [2,27]. As Mendelian genetics developed into molecular genetics, work in model systems attempted to identify the gene on the sex chromosome that was responsible for sex determination. The language of 'master regulator' was invented by Ohno [5], and came to dominate not only work on sex determination, but also developmental biology generally [28]. The term refers to a single gene, at the top of a pathway, that is not controlled by another gene, and that directs the differentiation of a cell type or tissue. However, all traits are controlled by multiple genes. Genes with larger effects will be easier to identify as Mendelian segregants compared with other genes that also contribute to the trait. Studies of laboratory mutants can bias discovery toward 'master regulators' because they focus on mutant alleles of large effect. This experimental mindset creates an intrinsic bias against identifying cases of PSD.

Genetic analysis of mutants

Genetic analysis in nonvertebrate model systems has also contributed to a particular perspective on the regulatory networks underlying sex determination. Analyses of epistasis among large-effect (Mendelian) laboratory mutants are often conducted under the assumption that developmental pathways are linear conduits for a signal [29,30] rather than homeostatic networks of positive feedback loops. A series of influential essays helped establish the idea that these linear pathways were built from the bottom up by the sequential addition of new master regulators, creating '**top-level sex determiners**' [31–33]. The concept of regulatory hierarchies helped to promote the idea that master sex determiners initiate particular pathways of male or female sexual development.

By contrast, work in vertebrate systems has led to a model of regulatory interactions that is distinctly nonlinear [34]. In Capel's view, two antagonistic regulatory networks battle for control of sexual development. One group of mutually reinforcing genes promote female development, while another group of genes promotes male development. Repressive interactions between these two networks are the basis of a bistable switch toward either male or female development (Figure 1). Thus, the 'master sex determiner' in mammals (SRY) is revealed to be a gene with a major effect in sex determination, but which does not sit at the top of a deterministic linear pathway. Rather, its transient expression early in development jumpstarts a SOX9 autoregulatory loop

pathways, then no gene can sit at the top of a regulatory hierarchy [31] and be an 'initial trigger' for sex determination. Sex determination in mammals has been described as being initiated by expression of SRY, but what controls the early pulse of expression of SRY? Another set of upstream regulators [35]. Turtles all the way up!

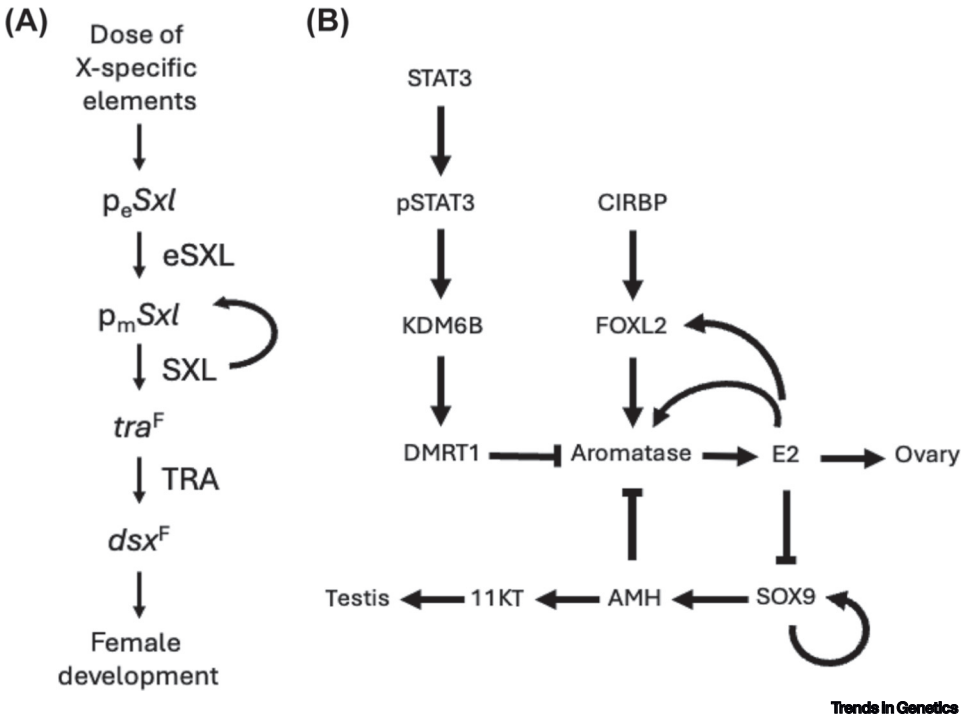

Figure 1. Contrasting perspectives on the regulation of sexual development. (A) The idea of top-down control through linear pathways (e.g., *Drosophila*). Note that the first step in the 'pathway' is a polygenic/quantitative switch, not a 'master regulator' [38]. (B) The idea of feedback loops leading to regulatory competition at a bistable switch (e.g., vertebrates). The relative dominance of the positive feedback loops (FOXL2–Aromatase–E2 in females vs. SOX9 auto-regulation in males) is mediated by reciprocal inhibition (AMH inhibiting aromatase and E2 inhibiting SOX9). Many other genes contribute to the establishment and maintenance of the feedback loops. Redrawn from [39] (A) and [40] (B).

that ultimately shifts the balance between two opposing networks [35,36]. The apparent permanence of sexual identity in mammals may reflect strong homeostasis of the alternative states of the bistable switch, rather than an irreversible determination by SRY [37].

Sex determination is not an event, but a stable state

Although we are most familiar with species that have separate sexes, many animals are either sequential or simultaneous hermaphrodites [41]. The existence of sequential hermaphrodites indicates that sex determination need not be a singular developmental decision in the life of an animal. Rather, sex may be maintained as a stable homeostatic state of a regulatory process that can be shifted by environmental or epigenetic factors. Bluehead wrasse (*Thalassoma bifasciatum)* usually develop as protogynous hermaphrodites. When the dominant male is removed from a patch reef, the largest female quickly transitions to male. The transition to male behavior is immediate, while the gonadal transition to producing functional sperm takes ~8 days [42]. The transition is clearly initiated by behavioral, not genetic information, shifting the otherwise stable pattern of gene expression in the female gonad [43].

The regulatory architecture of cells must usually follow a similar pattern. We recognize cell types that linger in a particular state because of the stability of a homeostatic regulatory network. Shifts from one state to another will usually require an input of information from outside the cell (e.g., hormone or intercellular signaling). Furthermore, development is not a linear process from zygote to adult, but a cycle across generations from zygote to zygote. The regulatory network must somehow re-establish a particular regulatory state (including a particular pattern of gene

expression) at the same point in the life cycle each generation. Thus, the idea that a particular gene is an 'initial trigger' for sex determination is unhelpful because it implies the process has a beginning and end. DNA sequence variants are just one factor that can nudge an ongoing developmental process from one stable state into another.

Sex at the level of cells, organs, and organisms

The developmental networks underlying sex determination can differ across organs within an organism [44] and the extent to which intercellular signaling is involved varies across taxa [45]. For example, in most eutherian mammals, *SRY* is important for gonadal differentiation within testis cells, which triggers hormonal signaling to the rest of the body to promote male developmental trajectories [46]. By contrast, somatic sex determination in birds is cell autonomous, meaning that sexual differentiation depends on the genotype of each somatic cell without relying on gonadal signals [47]. One consequence of cell autonomous sex determination is that gynandromorphs (i.e., sexual chimeras) can arise from sex chromosome aneuploidy early in development.

There is, in fact, a continuum of cell autonomous and hormonal sex determination both within organisms and across species, which undermines that idea that there is a single master sex determiner in any given species. In marsupials, for instance, a Y-linked factor (likely SRY) initiates testis differentiation, but the pouch, scrotum, and some other somatic tissues sexually differentiate according to the number of X chromosomes within each cell [48]. Sex determination depending on X chromosome number is reminiscent of *Drosophila*, although independently evolved. However, even in *Drosophila melanogaster*, which is a canonical example of cell autonomous sex determination, there are differences in how sexual identity is specified between germline and somatic tissues [49]. While sex determination in the *D. melanogaster* soma is cell autonomous, germline sex determination depends on both the sex chromosome complement of the germline cells and intercellular signaling from the surrounding soma [50]. Remarkably, this process is not even conserved across flies. In the house fly (*Musca domestica*), somatic sex determination is cell autonomous, while germline sex is controlled entirely by the sexual identity of the surrounding soma [51]. Moreover, many somatic cells in *D. melanogaster* do not express genes that are essential for somatic sexual differentiation and, therefore, do not 'know' their sexual identity [52].

These examples demonstrate that there may be no single sex determiner or mechanism of sex determination within a species. Instead, sexual differentiation is often regulated by a combination of cellular genotype (i.e., cell autonomous) and intercellular signaling (e.g., hormones), which can vary across tissues within an organism. Furthermore, the specific varieties of sex determination within species are poorly conserved even within a given taxon (e.g., Mammalia or Diptera). We are not arguing that this intraorganismal variation is PSD, *per se*, only that the molecular mechanisms of sexual development among tissues are diverse, implying that no single gene controls them all.

Polygenic inheritance need not produce intersexes

Another common misconception is that polygenic inheritance can only produce a continuous distribution of phenotypes. However, phenotypes depend on how continuous genetic variation is processed by the developmental system. Many developmental systems are able to process quantitative genetic and/or environmental variation to produce discrete outcomes [53]. Regulatory networks are selected to canalize outputs, which can occur through positive feedback loops that bring systems to alternative and relatively stable homeostatic states [54]. These networks provide a mechanistic basis for threshold traits, whereby the aggregate effects of alleles across multiple loci and/or environmental factors lead to alternative binary outputs (e.g., two

sexes) depending on whether a continuously distributed underlying variable is above or below a threshold.

The genetics of sexual development in zebrafish are illustrative. Wild populations frequently segregate a W allele of major effect on linkage group (LG) 4 [55,56]. During domestication of laboratory stocks, the Z allele was apparently lost [57]. Laboratory crosses still produce discrete sexes, but the sex ratios of individual families are highly variable and subject to environmental influence [58,59]. Considerable effort went toward mapping and characterizing the polygenic basis of sex determination in these lines [60,61]. There is no indication that these polygenic systems produce large numbers of intersexes. Almost invariably, individual embryos still develop as male or female because homeostatic feedback loops in the regulatory system canalize development into discrete sexes even without the presence of an allele of major effect.

Threshold model for sex determination

Quantitative geneticists have a well-developed theory for studying the genetic basis of threshold traits, integrating both genetic and environmental variation [25,62]. Figure 2 illustrates a threshold model for sex determination with a complex genetic basis. The x-axis, labeled liability (from the application of the model to human disease), represents the sum of genetic and environmental effects on a continuously distributed underlying variable. The developmental system produces discrete male or female phenotypes modeled as a step function in black. Below the threshold liability, the system produces males, and above the threshold it produces females. Intersexes are rare or absent despite the continuous distribution of liability because developmental canalization narrows the range of conditions that might produce intersex individuals. Numerous traits, from horn size in beetles to twinning rate in cattle, show discrete phenotypes despite polygenic inheritance [53,63].

The sex ratio of the population can change due to either a genetic or an environmental change that affects the liability. An increase in the environmental component of the liability (e.g., temperature), might result in selection on the genetic component of the liability to maintain an even sex ratio. Note that the threshold need not change in this scenario, only the components of liability (in this case, allele frequency).

Our understanding of the molecular basis of threshold traits lags behind the quantitative genetic model and will require detailed studies of the underlying regulatory networks. The mechanism for turning a quantitative input into a discrete output lies within the structure of the regulatory system, in which positive feedback loops and threshold-dependent responses together can produce qualitative 'switch-like' system behavior.

No true Scotsman

In our view, the simplest definition of PSD would be 'the presence of multiple genetic variants affecting sex determination in a population'. Schartl *et al.* propose a definition that appears to incorporate an element of development: 'the determination of sexual phenotype by the combined action of two or more genes at independently inherited loci in one individual' [12]. They further rejected some examples that they believe do not represent 'true' PSD. This rhetorical technique is known as the 'No true Scotsman' fallacy, which we deconstruct in the following section.

Elements of a common biochemical pathway

Schartl *et al.* suggest that multiple genes acting in a biochemical or developmental pathway in sex determination should not be considered polygenic [12]. Their basis for this definitional exclusion is that these genes are all under the control of a single master trigger. However, most textbook

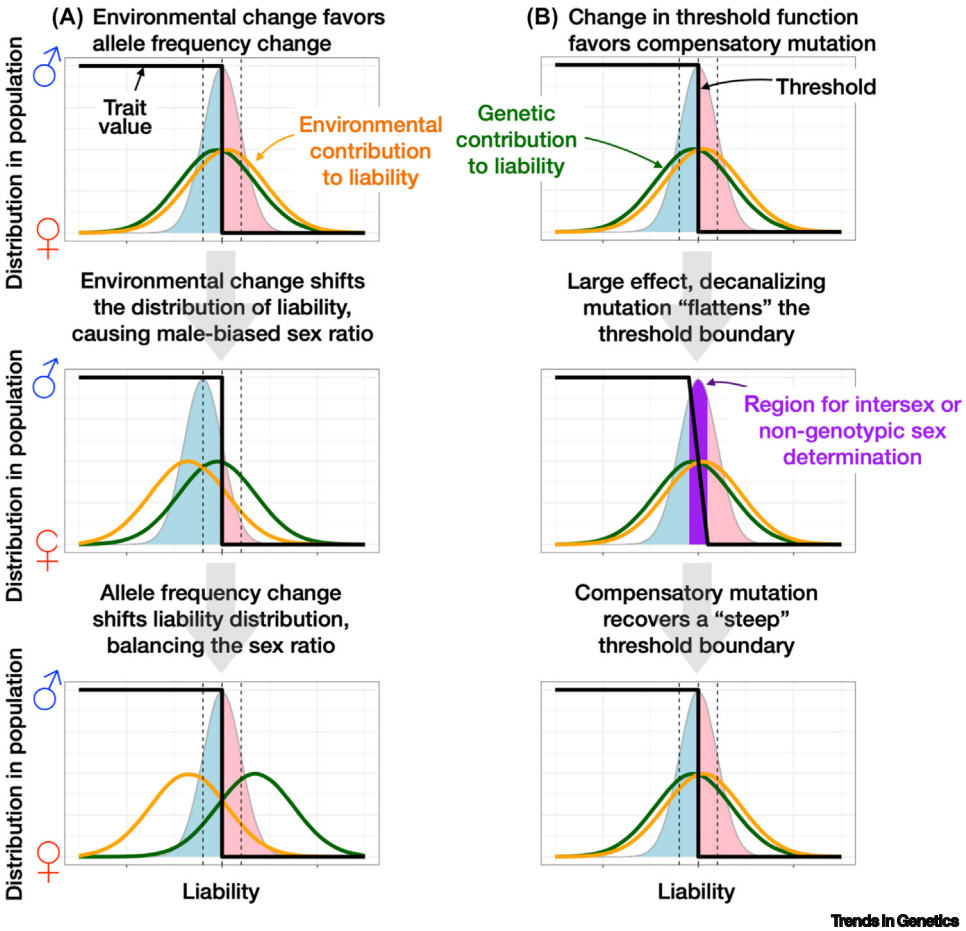

Figure 2. Threshold model for sex determination. In each plot, the x-axis represents the liability for the trait, averaging the genetic (green) and environmental effects (yellow) on an unspecified continuously distributed underlying variable, assuming no covariance between the two. The y-axis represents the probability of developing as a male. The black vertical line represents the threshold between male and female development. The blue/pink curve represents the phenotypic distribution of individuals in the population. (A) A change in the environment (e.g., temperature) changes the environmental component of liability, leading to a male-biased sex ratio. Selection on the sex ratio might then alter allele frequencies to return the population to an even sex ratio. (B) Mutations affecting the regulatory system can alter the shape of the threshold by decreasing the canalization of development, creating a region of liability values that result in intersexual phenotypes or nongenotypic sex determination. Selection against unfit intersex individuals may favor mutations that increase canalization.

examples of epistasis involve variants of enzymes involved in a putatively linear biochemical pathway. These clearly represent polygenic systems for the determination of blood type [64] or squash color [65]. It is difficult to imagine how multiple genes affecting sex determination would not, at some level, be a part of the same biochemical, developmental, or gene regulatory pathway or network.

Multiple alleles at the same genetic locus

Schartl *et al.* do not consider multiple alleles of a genetic locus (e.g., WXY) to represent PSD. This definition depends on how one defines a genetic locus. Many sex chromosomes harbor large regions of reduced recombination (e.g., inversions) containing hundreds of genes. While they segregate as a single Mendelian locus, it is conceivable that several genes within such a region might have effects on sex determination, while still genetically mapping to a single locus. The region around a new sex determiner (e.g., inside an inversion) would be expected to accumulate

alleles that increase the canalization of development (i.e., increase the genetic contribution to sex determination). The relatively broad regions within which many sex determiners are mapped often contain several plausible candidate genes for sex determination [66]. For the most part, experiments to distinguish the effects of variation at these loci have not been performed. For this reason, we suspect there are undiscovered transitions in the molecular mechanisms of sex determination [67]. Similar to supergenes, sex chromosomes may be inherited as a single locus containing multiple genes with complex molecular interactions affecting the phenotype [68,69].

Multiple sex chromosomes

Schartl *et al.* do not consider systems with multiple Y or multiple W chromosomes to represent PSD because they do not interact within the same individual to produce the phenotypic sex [12]. For example, in Malawi cichlids (described in the following section), the LG5 W chromosome is not usually found in the same individuals as the B chromosome that also acts as a W (Figure 3), because that would require mating between two females. From our perspective, both W chromosomes are segregating within a single population; thus, sex determination in these populations is polygenic.

PSD is real

Bull [25] identified three indicators of PSD: (i) a large variance in the sex ratio among families, (ii) paternal or maternal effects on family sex ratio, or (iii) a response to selection on sex ratio. To this we can add (iv) the genetic mapping of multiple factors affecting sex, either in experimental crosses or genome-wide association studies (GWAS). Table 1 lists just some of the many published examples of PSD in animals, focusing on vertebrate taxa. We discuss a few well-studied systems in the following sections.

Fish

African cichlids have figured prominently in recent research on the genetic basis of sexual development. Many rock-dwelling 'mbuna' cichlids from Lake Malawi segregate both an XY system on LG7 and a ZW system on LG5 [70]. An inversion on LG5 includes a dominant female-determining W, which is genetically linked to a dominant color polymorphism (orange-blotch) that provides females an alternative form of crypsis [99]. Many of these same species are also polymorphic for a B chromosome maintained by meiotic drive, which carries another epistatically dominant W locus [100]. Both W alleles are epistatically dominant to the LG7 XY locus. These Lake Malawi cichlids clearly represent systems of PSD involving at least three chromosomes with strong epistatic interactions among loci (Figure 3). These systems are segregating in many species and several genera of Lake Malawi mbuna, indicating that this PSD is not transitional but

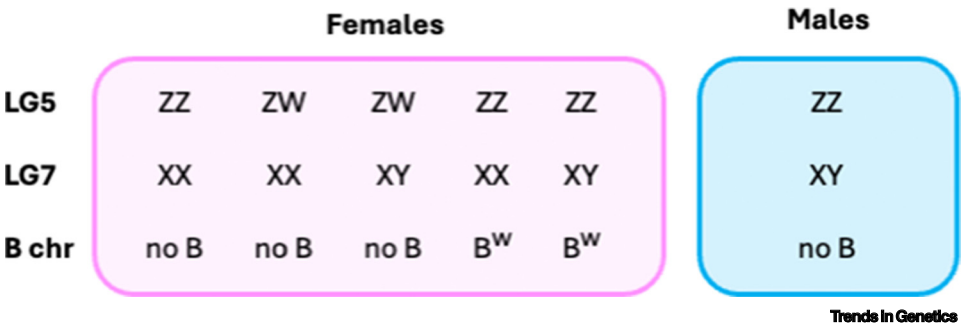

Figure 3. Polygenic basis of sexual development in mbuna cichlids of Lake Malawi. The sex of an individual is well predicted by the genotype at three loci: an XY system on linkage group (LG)-7, an epistatically dominant W allele on LG5, and an unpaired epistatically dominant B^W chromosome maintained, in part, by meiotic drive.

Table 1. Examples of PSD in animals

Family	Genus/species	Common name	Bull's indicators			Mapping	Refs
			Sex ratio variance among families	Parental effects on sex ratio	Sex ratio response to selection	Sex factors map to multiple locations	
Fish							
Cichlidae	*Maylandia* spp., *Labeotropheus* spp.	African cichlids	√	√		√	[70–74]
Poeciliidae	*Xiphophorus* spp.	Platyfish and swordtails	√	√			[3,75–77]
Moronidae	*Dicentrarchus labrax*	European sea bass	√	√	√		[22,78,79]
Mugilidae	*Mugil cephalus*	Flathead grey mullet				√	[80]
Danionidae	*Danio rerio*	Zebrafish	√	√		√	[57,58,61]
Adrianichthyidae	*Oryzias latipes*	Medaka	√	√		√	[81–83]
Amphibians							
Pipidae	*Xenopus tropicalis*	African clawed frog	√	√			[84]
Reptiles							
Emydidae	*Graptemys* spp.	Map turtle	√				[85,86]
Eublepharidae	*Eublepharis macularius*	Leopard gecko	√	√			[87]
Mammals							
Cricetidae	*Myopus schisticolor*	Wood lemming	√	√			[88,89]
Muridae	*Mus minutoides*	Pygmy mouse	√	√			[90–92]
Invertebrates							
Muscidae	*Musca domestica*	Houseflies	√	√	√	√	[93–96]
Harpacticidae	*Tigriopus californicus*	Tidepool copepods	√	√	√		[97,98]

has been maintained for thousands of generations [72]. Schartl *et al.* consider the phenotypic variation among the female genotypes of these cichlids to indicate intersexuality [12], but Moore *et al.* [73] are clear that there are only two gonadal sexes and no indication of reduced fertility in any genotype. Rather, the several female genotypes differ in aspects of behavior, pigmentation, and morphology, which may reflect different ecological or behavioral strategies, as is the case for the common cuckoo (*Cuculus canorus*) [101]. Many similar Y-linked alternative male strategies have evolved in fish [102–105].

Sex determination in fish of the genus *Xiphophorus* was studied in an extensive series of crosses in the premolecular era [76]. The southern platyfish (*Xiphophorus maculatus*) segregates W, X, and Y chromosomes, with the female-promoting activity of the W being dominant to the male-promoting activity of the Y. Schartl *et al.* do not consider this system polygenic because the three variants are on the same LG, but the genetic basis for this polymorphism is unknown [12] The distinction is semantic unless the differences are all at the same nucleotide. Another species, the Panuco swordtail (*Xiphophorus nigrensis*), has an XY system, but a recessive autosomal modifier results in XXaa individuals developing as males. In the Rio Choy population, the frequency of the autosomal modifier was estimated to be 0.26 [76]. Another way of looking at this system is that it is polymorphic for an XY and a ZW system, and the epistatic relationships are Y>W>Z>X. Two other species (*Xiphophorus nezahualcoyotl* and *Xiphophorus milleri*) segregate distinct Y chromosomes (Y and Y′) and an additional autosomal modifier [76]. Autosomal modifiers are also suspected in *Xiphophorus cortezi* and *Xiphophorus alvarezi* [77].

The European sea bass (*D. labrax*) meets all of Bull's criteria for identifying PSD. Crosses demonstrated a large variance in sex ratio among families and significant parental effects [22], with fast-growing individuals developing as females [106,107]. Selection for growth rate over three generations increased the female sex ratio by 20% [79]. Mapping in experimental crosses revealed different genetic architectures within and among wild populations and between four and nine quantitative trait loci (QTL) for sex in each population [78].

A GWAS of the flathead grey mullet (*Mugil cephalus*) identified a missense haplotype of follicle-stimulating hormone receptor (*fshr)* affecting sex determination [80]. However, the male-biasing (Y) haplotype is incompletely penetrant, with ~10% of females carrying the male haplotype. The frequency of the male haplotype is also highly variable across populations. Some 30 other genome assembly contigs contain sex-associated variants, but the genome assembly is not contiguous enough to conclusively demonstrate PSD. These observations suggest a contribution of minor genetic loci and/or environmental factors to sex determination in this species.

The search for a master sex determiner in the laboratory zebrafish (*Danio rerio*) went on for years and identified several genes with quantitative effects on sex determination [58–61]. Finally, new collections from the wild revealed a relatively old ZW sex chromosome on LG4 [56]. During domestication, laboratory populations were apparently fixed for the ancestral W chromosome [57], and selection favored increases in the frequency of male-promoting alleles at other loci. Likewise, the deliberate experimental removal of DMY on Chr1 from laboratory populations of medaka (*Oryzias latipes*) helped reveal another sex-determining locus on Chr18 [83]. While it can be argued that these 'minor' sex determiners in laboratory populations are irrelevant when the major sex determiners are present, they nevertheless indicate a large hidden pool of genetic variation for sex determination. They also highlight the strong canalization of development into discrete sexes even in the absence of alternative alleles at the 'major' sex determiner.

Other vertebrates

The tetraploid African clawed frog (*Xenopus laevis*) and its close relatives have been described as a ZW system segregating DM-W, a duplicate of DMRT1 transposed to Chr2 [108,109]. Its diploid congener *Xenopus tropicalis* lacks DM-W and instead segregates WXY alleles on Chr7 [84]. The Y chromosome evolved from the ancestral Z chromosome, and all three alleles segregate within natural populations. However, the molecular differences responsible for sexual phenotype have not been determined [110]. The great diversity of sex chromosomes in frogs [111] suggests further examples of PSD may be hidden in this clade.

Reptile species with suspected PSD include map turtles (*Graptemys* sp.) and leopard geckos (*Eublepharis macularius*). Both taxa have temperature-dependent sex determination (TSD), but experimental evidence has found variation in sex ratios among families consistent with PSD [25,86,87].

Several rodents have polygenic sex systems [112]. The wood lemming (*Myopus schisticolor*) has an XY system, but also X*Y females, in which the X* chromosome suppresses the Y chromosome [88]. Nondisjunction in X*Y females produces X*X* females, leading to a sex ratio highly biased toward females. A similar X, X*,Y system evolved independently in the African pygmy mouse (*Mus minutoides)*. This polygenic system is maintained, in part, by chromosome drive [92]. In both species, the X* chromosome can be distinguished cytologically and is effectively a W chromosome that is dominant to the Y.

Invertebrates

The house fly (*Musca domestica*) has one of the best characterized PSD systems of any animal [113]. In house fly populations, a dominant male-determining locus (*M* or *Mdmd*) can be found on any of the six pairs of chromosomes [96], meaning that every chromosome can be an X–Y pair. *M* causes the *M. domestica* ortholog of *transformer* (*Md-tra*) to be spliced into a nonfunctional isoform, which initiates a male developmental trajectory [94]. In the absence of *M* in the genome, *Md-tra* is spliced into an isoform that encodes a protein that initiates a female developmental trajectory. In addition, a dominant allele of *Md-tra* ($Md\text{-}tra^D$) segregates in house fly populations, which initiates female development even if there are copies of *M* in the genome. Therefore, the chromosome carrying $Md\text{-}tra^D$ is a W chromosome. The house fly PSD system has been evolutionarily stable for as long as it has been observed in natural populations (decades), and possibly longer [95]. Selection pressures have been identified that can maintain stable PSD within and across house fly populations [114,115].

The copepod (*Tigriopus californicus*) lives in the highly variable environment of intertidal splash pools with a complex metapopulation structure. It has a polygenic system of sex determination with moderate heritability [98,116]. QTL mapping identified at least six loci accounting for 19% of the variance of the offspring sex ratio [97].

Whether you consider PSD rare or widespread may depend on your perspective. Rather than trying to decide whether PSD does or does not occur, it might be more helpful to document its distribution and prevalence across various clades. Clearly more work is needed to determine the abundance and distribution of PSD in metazoans. Given its broad phylogenetic distribution, we predict that its frequency will be determined by ecological factors rather than by historical accident.

Prevalence of PSD has been underestimated

Despite this relatively long list of polygenic systems, the actual prevalence of PSD has likely been underestimated. Characterizing the loci affecting sexual development shares the difficulties of mapping any complex trait. Detection of individual loci in a genome scan depends on the frequency of alleles in the population, the interactions among alleles at a locus (dominance), the interactions among alleles at different loci (epistasis), the magnitude of the effect of each allele, and the particular environment in which these effects are measured.

Experimental designs to detect loci affecting sexual development can be broadly divided into two categories. The first is the mapping of QTL associated with sex in laboratory crosses [117–119]. However, such studies typically sample a small number of parents from the population (so that some sex-determining alleles in the population are not sampled), and frequently do not genotype enough progeny to detect loci with minor effects. The second approach is a GWAS for sex in a sample of unrelated individuals [80,120,121]. Given that the cost of DNA sequencing usually prohibits separate genotyping of each individual, sequencing is often performed on pooled DNA, comparing a group of 20–30 males to a similar sample of females.

These experimental designs are typically executed without the statistical power to detect multiple loci affecting sexual development. We modeled our ability to detect PSD in a typical pooled sequencing experiment. We were specifically interested in the effects of epistasis and modeled two patterns of epistatic interaction between an XY and ZW locus (Figure 4). The criteria for detection are that one allele (e.g., Y) is present at a frequency between 0.3 and 0.7 in one sex and at a frequency <0.1 in the other sex [122]. The genotype frequency space in which both systems are detected is small, and largely corresponds to regions where sex ratios are very

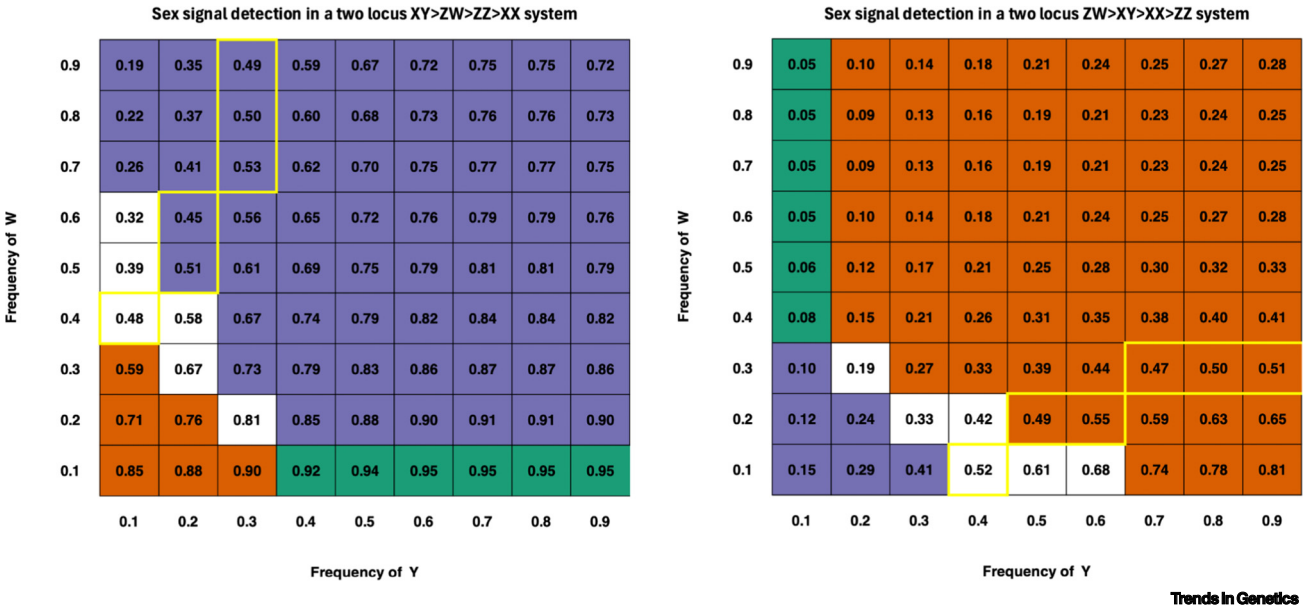

Figure 4. Experimental power in a typical pooled sequencing experiment. The axes for each plot are the frequency of the Y and W alleles at two loci affecting sex determination. The numbers in each cell are the expected sex ratios for the population. Two patterns of epistatic interaction are modeled. The probability that the Sex_SNP_finder pipeline [122] will detect the sex loci is given by the background color code: green, both the XY and ZW signal will be detected; purple, only the XY signal will be detected; orange, only the ZW signal will be detected; white, neither system will be detected. Most natural populations will fall in the regions with near-equal sex ratios (yellow border).

uneven, a situation that is unlikely to persist in nature [123]. Within the frequency space where sex ratios are near 0.5, most pooled sequencing experiments will detect only one locus, and many will not detect either locus. While this particular analytical pipeline is designed to identify sex-patterned SNPs, the power of any statistical approach for detecting sex loci will be limited by both the effect size and the frequency of the allele in the population.

Experiments with high statistical power have revealed multiple sex determiners within natural populations. A study of another cichlid fish (*Astatotilapia calliptera*) from Lake Masoko performed full genome sequencing on each of 647 individuals collected from the wild [74]. GWAS identified a highly significant XY locus on LG7, which was found to correspond to a 20-kb duplication that included *gsdf*. However, this duplication was not found in 15% of the males. A second round of GWAS on these 15% identified a 700-bp insertion near *id3* on LG19. A third round of GWAS on the 5% of males that did not carry either of these mutations identified a 5-kb insertion 2.5-kb upstream of *gsdf* on LG7. Thus, the cichlid population in this crater lake, which is only 700 m in diameter and believed to have formed just 50 000 years ago, has a PSD system that is segregating at least three distinct Y chromosomes. Given that most published studies have far less experimental power, we conclude that the prevalence of PSD has been underestimated.

Theory does not preclude PSD

Population genetic theory has identified multiple biologically realistic conditions that can maintain evolutionary stable PSD. While Schartl *et al.* [12] discussed models that suggest PSD is unstable [25,124,125], these represent a limited subset of the theoretical studies of PSD. Here, we present a more thorough review of theoretical models of PSD, describing how both the structure of the model and the selection pressures considered can contribute to whether PSD is stable.

Modeling PSD

Models of PSD typically take two different forms. In the first approach, implemented by Bull [25,124] and Rice [125], the causal loci and alleles are not specified. Sex is modeled as a threshold trait (Figure 2) that is determined by a liability score (arising from a combination of genotypic and environmental effects) and a threshold value that is the boundary between male and female development. Rice [125] simplified this further by removing the environmental component and using a single parameter for the probability that a zygote develops into a male or female based on the parental genotype, an approach that has been implemented in subsequent work [126]. In the second approach, specific genotypes at a finite number of designated loci combine to determine sex [115,127–129]. This second approach considers dominance and epistatic relationships of genes and alleles, rather than modeling PSD as a threshold trait. In both approaches, the evolutionary stability of PSD is examined by considering whether new sex-determining factors can invade a population and/or if there is a stable equilibrium that includes two or more SD loci.

When PSD is not stable

Some theoretical analyses of PSD have indeed identified conditions of evolutionary instability. For example, Rice [125] showed that a threshold trait form of PSD is susceptible to invasion and replacement by a novel dominant male determiner (Y) that is epistatic to the ancestral PSD system if it is genetically linked to a male-beneficial, sexually antagonistic allele. In addition, van Doorn and Kirkpatrick [127,128] identified multiple scenarios in which a monogenic XY sex chromosome system could be replaced by a different monogenic XY or ZW system, with PSD only existing as an unstable intermediate between the monogenic equilibria. While these results identified specific cases in which PSD was not evolutionarily stable, they did not demonstrate a general instability of PSD.

Selection on sex ratios

Explorations of the interplay between sex determination and population sex ratios have revealed insights into how PSD can be maintained at a stable equilibrium. Many populations have approximately equal numbers of males and females, although various ecological factors and reproductive strategies can favor sex ratios that deviate from 1:1 [130,131]. It has been shown by Bull [25] and others that selection on sex ratios, both toward and away from the 1:1 balance, can drive the evolution of sex determination (reviewed in [132]).

Most relevant here is work by Bateman and Anholt [126], which showed that PSD can be maintained in a metapopulation by selection on sex ratios. They modeled PSD in the copepod *T. californicus* as a temperature-dependent threshold trait, but assumed that the liability arises from the genotype only, while the threshold shifts according to temperature. Using sinusoidal oscillation of the threshold value to model seasonal fluctuations in temperatures, they found that PSD could be maintained for long periods across metapopulations via migration. This is a specific example of the more general phenomenon of adaptive tracking of fluctuating selection pressures, which can maintain genetic variation in natural populations [133,134]. The generality of this phenomenon suggests that PSD could be maintained broadly through fluctuating selection pressures that affect the liability (or threshold) of sex determination.

Selection on linked alleles with sexually antagonistic fitness effects

Models of PSD with specified loci and alleles have consistently shown that sexually antagonistic selection can maintain PSD. The first of these modeled the platyfish *X. maculatus*, which segregates an X, a male-associated Y, and a female-associated W on the same chromosome [135]. In this system, the X, Y, and W chromosomes can be maintained as a stable polymorphism if the Y and W each carry a male or female beneficial (respectively) sexually antagonistic allele,

and those alleles are in tight genetic linkage with the sex-determining loci on those chromosomes [129,135]. Similarly, two different male-determining Y chromosomes can be maintained as a stable polymorphism if they have comparable male-beneficial sexually antagonistic fitness effects [127].

Recessive deleterious alleles are expected to accumulate on Y chromosomes because they are protected from selection in heterozygous XY males. A PSD system in which there are separate XY and ZW chromosomes can be maintained in a population if the male-determining Y harbors recessive deleterious alleles and the female-determining W carries female beneficial sexually antagonistic alleles, or vice versa for the Y and W [128]. Similarly, overdominant fitness effects of Y chromosomes (i.e., heterozygote advantage) can maintain a PSD system with a W chromosome and multiple Y chromosomes [115], and those overdominant effects could arise if the Y has dominant male-beneficial alleles and recessive deleterious alleles. These may not be stable equilibria because selection in ZW;YY females, combined with recombination between Y chromosomes, will allow purging of deleterious Y-linked alleles from the population, allowing fixation of the YY genotype and creating a monogenic ZW system [128]. Nonetheless, the period of PSD is very long with stable frequencies of Y and W chromosomes, and even longer if recombination is suppressed between the male-determiner and male-beneficial alleles on the Y chromosome, which would create the appearance of stable PSD if observed in a natural population.

The dominance of the sex-determining locus has further effects on the maintenance of PSD. A stable XY;ZW system is possible if the male-determining activity of the Y is incompletely dominant [128]. While it is tempting to dismiss incomplete dominance as biologically unrealistic, it could instead be envisioned as an incompletely penetrant, decanalized, or environmentally sensitive male determiner. Environmental factors overriding the male-determining activity of a Y chromosome have been observed in green frogs (*Rana clamitans*) [136], demonstrating that such a scenario is feasible.

Concluding remarks

There is much more to learn about the genetic and developmental mechanisms of sex determination, and how these mechanisms evolve (see Outstanding questions). It is clearly interesting to ask how common PSD is, and whether its incidence varies among lineages. To date, most studies have not been designed to look for PSD, especially if there are loci with smaller effects. We do not find it helpful to define away the existence of PSD. Sex determination may often have a complex genetic basis, but the phenotypes are canalized by the developmental process into discrete male and female phenotypes. Intersexes are rare, but PSD may be common.

Outstanding questions

How can we efficiently characterize the genetic architecture of sex determination in more species?

How many genes participate in the developmental process of sexual differentiation? How many can become Mendelian factors in sex determination?

How do developmental processes canalize development into discrete sexual phenotypes?

What are the positive feedback loops that maintain male or female states in vertebrates?

What are the negative feedback mechanisms that contribute to the bistable switch between male and female development?

What are the evolutionary forces that maintain genetic variation for sex determination?

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Declaration of interests

No interests are declared.

Resources

[i]https://en.wikipedia.org/wiki/Turtles_all_the_way_down

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