Sexual
Development

Sexual Development and the Evolution of Sex Determination

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Abstract

The evolution of sex had profound consequences for life on earth as it brought about the origin of males and females. Yet understanding the amazing variety of mechanisms employed by organisms to produce males and females has evaded scientific explanation. Developmental biology provides essential insight to understand how the sexes are formed, and how the regulatory network underlying sexual development changes over evolutionary time. This is a critical step in revealing why the diversity of mechanisms arose in the first place. Here I present significant reciprocal contributions made by evolutionary genetics and developmental biology that bear on this question. In particular, I explore how changes in the timing of events during development can be viewed as heterochronic changes that alleviate the strong selection on the master trigger of male and female sexual development, enabling the evolutionary transitions between genotypic and environmental sex determination.

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The origin of sexual reproduction, or the combination of genetic material from two parents in the offspring, marked a paramount moment in the history of life on earth. This event caused the evolution of a multitude of traits, including the evolution of mating types, mate discrimination, anisogamy, sex allocation and sex ratios, among others [Williams, 1975; Barton and Charlesworth, 1998]. The evolution of anisogamy, or the production of gametes of different size (large and nutrient rich eggs versus small and highly mobile sperm), defines the origin of male and female functions which are found in separate individuals in dioecious species and within the same individual in hermaphroditic taxa [Williams, 1975].

For millennia humankind has pondered how males and females are produced in species with separate sexes. It was not until the early 1900s that an answer to this question was found by the discovery of sex chromosomes and their association with sex determination [McClung, 1902; Stevens, 1905; Wilson, 1905; reviewed in Brush, 1978]. Because all dioecious organisms must produce males and females, it is tempting to assume that the mechanism underlying the production of such conserved phenotypic outcomes is equally conserved. But a century after the seminal finding of sex chromosomes, it is now recognized that sexually-reproducing organisms employ an astonishing diversity of mechanisms to produce males and females, ranging from systems under strict genetic control (GSD) to systems under strict environmental control (ESD) [Bull, 1983; Solari, 1994; Valenzuela et al., 2003; Valenzuela and Lance, 2004]. GSD mechanisms include highly dimorphic or undifferentiated sex chromosome systems in male (XX/XY) or female (ZZ/ZW) heterogamety, which is the most common mechanism among animals, but other systems are known as well [Bull, 1983]. While XX/XY is found in all mammals with some notable exceptions [e.g. Grützner et al., 2004; Just

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et al., 2007], and ZZ/ZW is found in all birds, both systems coexist among amphibians, fish and reptiles, and co-occur with ESD in fish and reptiles [Bull, 1983; Solari, 1994; Valenzuela and Lance, 2004]. Contrasting sex determining systems of male and female heterogamety can be found among closely related species [e.g. *Oryzias* spp., Takehana et al., 2007; Reptilia, reviewed in Valenzuela and Lance, 2004], or even among populations of the same species [e.g. *Rana rugosa*, Ogata et al., 2002]. This astounding diversity requires both a functional and an evolutionary explanation. Indeed, the recurrent transitions among sex determining mechanisms remains one of the most fascinating enigmas in evolutionary biology.

The difficulty in explaining the repeated evolutionary switch among sex determining mechanisms emerges from several observations. First, GSD involves a suite of correlated traits seemingly under strong selection. The same is true for TSD. This implies that both mechanisms are strongly adapted for the particular ecological conditions in which they are found. Thus, if GSD and TSD represent different peaks in the adaptive landscape [Wright, 1932] and both have evolved repeatedly from each other, the question that follows is how were species able to move between adaptive peaks? One possibility for such a shift to be accomplished is for selective pressures to somehow be relaxed, such that taxa can cross the adaptive valley and move from one adaptive peak to the other. I argue that when viewed from a developmental perspective, significant insights and predictions about the evolutionary process that allows transitions between mechanisms can be made. This happens because in order to understand why the recurrent evolutionary transitions between sex determining systems have occurred, it is necessary to understand how these mechanisms work. Describing the existence of GSD by sex chromosomes or of ESD by factors such as temperature (TSD) provides no satisfactory answer if one is interested in understanding how *exactly* males and females are produced. This question is better addressed by developmental biology. A definitive response will be found in the detailed study of the events that take place during the development of model organisms, and in the comparative analysis across model and non-model taxa, which allows testing the breadth or limitations of the generalizations discovered in the model systems.

Here I will argue that increased knowledge from a developmental perspective helps our understanding of sex determination at several other levels of organization, from molecular evolution within the gene networks underlying sex production, to the regulation and function of those networks, to the evolution of sex ratios and the dynamics of populations. Thus, by understanding the *how* at the proximate level, we should also get closer to answering *why* males and females are produced by such diverse mechanisms. In this article I will concentrate mainly on primary sexual development in vertebrates, or the determination and differentiation of the gonads.

The Basics

Sexually reproducing organisms form by the union of a male and a female gamete at fertilization and the subsequent formation and growth of tissues and organs that gives rise to a fully-developed individual (fig. 1). At the developmental level therefore, scientists are concerned with cell determination and cell differentiation in order to decipher how a unicellular zygote becomes an adult male or female. A great deal is known about the control of these developmental steps and the involvement of organizers, fields and morphogens during ontogenetic changes [Gilbert, 2000; Carroll et al., 2005]. Gonadogenesis in particular, requires a delicate balance between short and long-range signaling molecules that are required for the formation of the bipotential primordia, the determination to a male or female developmental fate, and the ensuing differentiation into testis or ovaries [Kim and Capel, 2006]. These events involve a gene regulatory network composed of numerous elements common to all vertebrates [Place and Lance, 2004], but whose initial triggers vary between and within GSD and TSD systems.

Unambiguous Definitions

Although both evolutionary and developmental biology use the term 'sex determination', the meaning in these two fields is not always the same. In developmental biology, *sex determination* refers to the commitment of cells and tissues to the male or female developmental fate, which is followed by *sex differentiation*, or the development of specialized male and female cells, tissues, and organs (e.g. testis or ovaries). By contrast, evolutionary geneticists are interested in the level of inheritance, or how to distinguish the way in which maleness or femaleness is transmitted between generations, such as by different sex chromosome systems [Bull, 1983]. Thus, it is important to notice that in ecology or at the level of inheritance, the term *sex determination* is used to distin-

Fig. 1. Ontogenetic events during the life cycle of organisms. **a** General steps from fertilization to reproduction. **b**, **c** Contrast of the timing of events between organisms with genotypic sex determination (**b**) and environmental sex determination (**c**). Chronologically, differences between GSD and TSD start at fertilization, marked by the lack of sex identity in TSD zygotes. This causes the delay in the realization of primary sex ratios in TSD compared to GSD. Note how the sex determination is expanded and overlaps with sex differentiation in TSD.
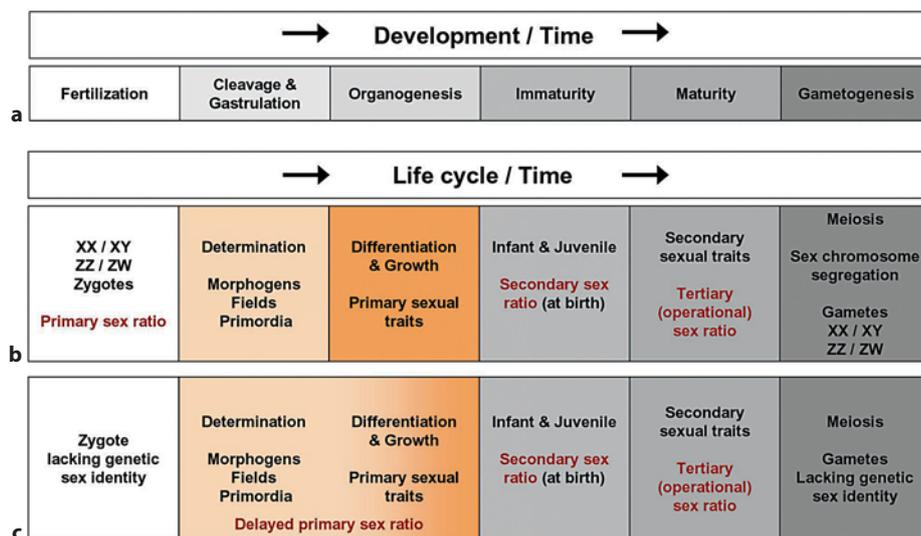
guish the mechanisms found in organisms possessing sex chromosomes whose sexual identity and fate can be identified at conception (GSD), from the mechanisms found in organisms lacking any consistent heritable genetic difference between the sexes and whose sexual identity and fate is established plastically some time after fertilization by an environmental factor (ESD, TSD), and from intermediate GSD mechanisms susceptible to environmental effects of various kinds [Bull, 1983; Valenzuela et al., 2003; Sarre et al., 2004]. To enable the clear communication between these fields of inquiry, a comprehensive definition of *sex determination* should be used, i.e. 'the process of irreversible commitment to the male or female developmental fate, which can be initially triggered by genetic factors such as those contained in sex chromosomes (GSD) or by environmental factors (ESD) such as temperature (TSD)'.

Developmental Heterochrony between GSD and TSD

Some noticeable discrepancies between GSD and TSD become evident when one examines the chronology of developmental events under these two mechanisms (fig. 1). Probably the most distinctive chronological difference in development between GSD and TSD is in the timing of commitment to the male or female developmental fate. Primary sex determination in GSD males such as mammals occurs by the expression of *Sry*, which induces the consequent differentiation of Sertoli cells

[Kim and Capel, 2006]. After these supportive cells have undergone specification, other testis specific cells differentiate. In females (XX), primary sex determination is not as well understood but ovary-specific gene activity also occurs earlier than morphological differentiation [Nef et al., 2005]. Less is known about the control of gonadogenesis in ZZ/ZW vertebrates such as birds, and the involvement of *Dmrt1* as a master switch similar to *Sry* in mammals remains uncertain [Morrish and Sinclair, 2002; Zhao et al., 2007]. Although the paucity of data from TSD taxa such as reptiles is far greater [Place and Lance, 2004], existing information permits important comparisons to be made. For example, shift incubation experiments have revealed that temperature affects sex determination during a specific window of time during embryonic development (thermosensitive period or TSP) [Valenzuela and Lance, 2004]. The TSP encompasses roughly the middle third of incubation in oviparous TSD reptiles, which is longer (both in absolute and relative terms) than the period needed for sex determination in mammals or birds [Pieau, 1996; Morrish and Sinclair, 2002].

More importantly, unlike in GSD where sex differentiation appears to follow well demarcated sex determination events, in TSD sex differentiation starts during the TSP before sex determination is completed [Pieau, 1996], such that there is a significant overlap between the two processes [Pieau et al., 1999] (fig. 1). In evolutionary terms, this means that considerable heterochronic changes [Alberch et al., 1979] in these developmental steps separate GSD mammals and birds from TSD reptiles. Since in general *determination*, or the irreversible commitment

to a developmental fate, is preceded by the *specification*, or the labile stage where the commitment is still reversible [Gilbert, 2000], the heterochronic differences between these groups seem to be caused in part by a longer period of specification in TSD reptiles (the TSP) compared to that of GSD mammals and birds. Additionally, a more labile onset of gonadal differentiation in TSD taxa that can occur prior to the irreversible gonadal determination (while the TSP is still active) renders the events of the developmental sequence less discrete than in GSD mammals and birds. Furthermore, sex determination may not be fully irreversible in TSD species even after the end of the TSP [Dorizzi et al., 1996]. One possible explanation for the relatively early onset of gonadal differentiation in TSD reptiles (i.e. prior to the irreversible commitment to a particular sexual fate) may be that delaying the onset of differentiation until the end of determination is disadvantageous given the longer absolute time that the entire process takes in these taxa, such that a relatively earlier start of differentiation might be adaptive. Interestingly, although GSD reptiles appear to follow the same general trajectory of differentiation as TSD reptiles [Raynaud and Pieau, 1985] gonads of GSD taxa are committed to their sexual fate at an earlier developmental stage than in TSD close relatives [Greenbaum and Carr, 2001; Neaves et al., 2006].

Additional Discrepancies between GSD and TSD

Other important discrepancies between GSD and TSD also exist. Chronologically, the first difference between GSD and TSD mechanisms appears at fertilization. Indeed, the notion of primary sex ratio, or sex ratio at conception, is a construct that does not apply to pure TSD species because genotypic sex does not exist and the sex of individuals is unknown at fertilization given that no consistent genetic differences exist between TSD males and females [Valenzuela et al., 2003]. A special case is represented by species possessing sex chromosomes and whose sex is reversed or their sex ratios distorted by environmental factors [e.g. Robert and Thompson, 2001; Shine et al., 2002]. In those instances one can distinguish the genotypic sex (by the presence of sex chromosomes) from the phenotypic sex of individuals and whether or not they are consistent [Valenzuela et al., 2003]. Thus, primary sex ratio does exist in such taxa. On the contrary, in TSD species the primary sex ratio is non existent at conception or its realization is delayed until some time during development but before birth/hatching, specifi-

cally, until the end of the thermosensitive period. The lack of sex identity at fertilization in pure TSD species may delay early developmental events that are sexually dimorphic in GSD species, such as the expression of important genes and other physiological traits with fitness consequences [e.g. Gutierrez-Adan et al., 2006; Morton et al., 2007].

Likewise, another difference between GSD and TSD exists with respect to the notion of secondary sex ratio, or sex ratio at birth. Like the concept of primary sex ratio, secondary sex ratio does not have a ubiquitous applicability. Particularly in fish, sex determination and differentiation is not evident until after posthatching [Devlin and Nagahama, 2002] such that in TSD fish in which sex identity is lacking prior to sex determination, sex ratio at birth is non existent or its realization is delayed for weeks or months after hatching. Additionally, fish in general possess more labile mechanisms of sex determination and a wider array of sex differentiation trajectories than other vertebrates [Devlin and Nagahama, 2002].

At maturity these heterochronic differences disappear as the notion of the tertiary sex ratio, or the operational sex ratio of adults, applies to all vertebrates, from GSD to TSD. Even in sequential hermaphrodites that mature first as one sex and suffer a complete reversal to the opposite sex, such as occurs in some fish [Sadovy and Shapiro, 1987], one could examine the existing tertiary sex ratio of a population at any given point in time. The difference, however, is that in sequential hermaphrodites the tertiary sex ratio changes over time by the sex reversal of adults in addition to changes due to external factors such as sex-specific mortality that also affect other non-hermaphroditic species.

Finally, at gametogenesis we find that again GSD and TSD mechanisms differ fundamentally (fig. 1) because GSD gametes possess sex chromosomes and thus contain information about sex identity (e.g. X-bearing versus Y-bearing sperm) whereas TSD gametes do not. Whether GSD gametes carry one or the other sex chromosome (X/Y or Z/W) can have important fitness effects, an attribute that has been proposed as a mechanism by which GSD taxa may adaptively manipulate sex ratio [reviewed in Rosenfeld and Roberts, 2004; Alonso et al., 2006].

Implications

The appreciation of differences and similarities between GSD and TSD mechanisms at various levels of organization makes evident how developmental biology

can provide invaluable insight about evolutionary genetics and vice versa. For instance, advances made by evolutionary genetics about sex determination dealing with how sex is inherited across generations [Bull, 1983] help to understand the inheritance of the initial trigger of the male and female developmental programs. In so doing, this knowledge helps to resolve the composition, regulation, and evolution of the gene networks underlying sexual development. On the other hand, when developmental biologists discover a master trigger of male and female development, its mapping to a specific genomic location reveals the identity of the pair of sex chromosomes. This is particularly helpful for GSD species whose sex chromosomes lack gross heteromorphism and pass undetected by conventional cytogenetic techniques, and for which even high resolution methods [e.g. Ezaz et al., 2006; Kawai et al., 2007] may fail in identifying the sex chromosomes.

Thus, the merging of these two fields of research that resulted in the advent of modern evolutionary developmental biology has enabled important discoveries. For instance, up until the late 1990s, all evidence indicated that the well characterized GSD mechanisms of model invertebrates such as *Drosophila* and *Caenorhabditis elegans* had independent evolutionary origins than those of vertebrates, as no element was known in common between their underlying gene regulatory networks [Capel, 1998]. Furthermore, the realization that sexual dimorphism is ancient, dating back to the last common ancestor of coelomate bilaterians, posed a problem in light of how diverse the genetic control of sex specification is known to be [Haag and Doty, 2005]. And it was the finding of the DM gene family across vertebrates and invertebrates [Raymond et al., 1998, 1999; Miller et al., 2003] showing that their sex determining mechanisms may derive from a shared common ancestor that helped to solve this apparent dilemma [Haag and Doty, 2005]. A similar question about homology and common ancestry relates to the sex chromosome systems found in vertebrates (XY or ZW). Ohno [1967] speculated that mammalian and bird sex chromosomes might derive from a common pair of autosomes, but evidence until now contradicted this idea and supported the widely-accepted notion that the mammalian XY and avian ZW systems evolved independently from different autosomal pairs [Fridolfsson et al., 1998; Nanda et al., 1999; Ellegren, 2000; Ezaz et al., 2006; Kohn et al., 2006]. Furthermore, avian ZW and serpent ZW systems also appear to have independent evolutionary origins [Kawai et al., 2007], counter to Ohno [1967]. However, new data has challenged this widely held view

as amphibian orthologs of genes found in avian and mammalian sex chromosomes map to a single chromosome in amphibians [Smith and Voss, 2007], supporting a common ancestry for mammalian XY and avian ZW systems.

Most importantly however, the discovery of the conserved DM gene family and of the purported common autosomal origin to vertebrate sex chromosomes opens a new conundrum. Namely, why has the genetic control of sex specification [Haag and Doty, 2005] and the chromosomal system of inheritance of the initial trigger of that specification, diverged so much from its common ancestor? The answer to this question will come by integrating data obtained from evo-devo and evolutionary ecology studies.

For instance, genes of the network that regulates sexual development suffer higher rates of molecular evolution relative to other non-sex related genes [Civetta and Singh, 1998]. Additionally, elements of these pathways were gained or lost and their connections or mode of action altered in some lineages [Haag and Doty, 2005; Valenzuela and Shikano, 2007; Valenzuela, 2008]. The high rates of molecular evolution of sex related genes may be the result of sexual selection acting at the population level, and thus they would be the genetic signature of a process that could result in speciation [Civetta and Singh, 1998; Borge et al., 2005]. On the other hand, sex chromosomes that bear the master trigger for sexual development in GSD taxa have another powerful property. Namely, the initial step of sex chromosome evolution from a pair of autosomes by the appearance of a sex-determining mutation is followed by the accumulation of additional mutations with sex-specific advantage and by decreased recombination [Bull, 1983; Ohno, 1967; Charlesworth, 1991]. This process may generate two morphologically distinct sex chromosomes that differ in their pattern of heterochromatin accumulation and deletions, and may cause the degeneration of the non-recombining heterogametic sex chromosome (Y or W) [Ohno, 1967; Vallender and Lahn, 2004; Waters et al., 2007] and even its ultimate extinction [Graves, 2002, 2006]. A new pair of sex chromosomes may be formed de novo by the reiteration of the same process [Charlesworth et al., 2005; Just et al., 2007], or by the translocation of an ancestral sex determining gene onto an autosome resulting in a neo-sex chromosome that can become fixed in populations by selection acting on sexually antagonistic genes linked to it [Van Doorn and Kirkpatrick, 2007]. Furthermore, the hemizygosity and reduced recombination characteristic of sex chromosomes is predicted to affect the

Fig. 2. Heterochrony in the developmental windows of sex specification, determination and differentiation between temperature-dependent and genotypic sex determination, causing a significant overlap of these windows in TSD and a more sequential development in GSD.
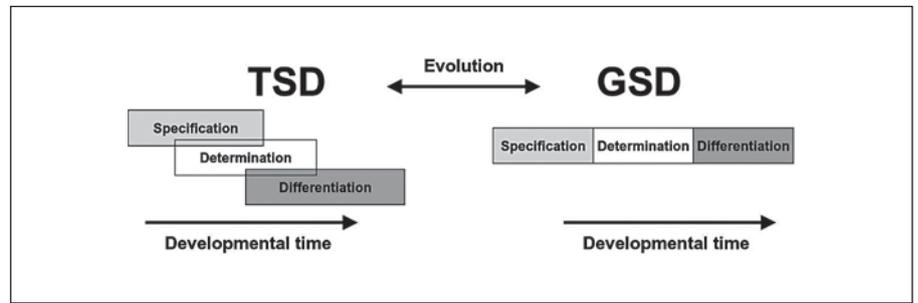
mechanism of natural selection and consequently the patterns of molecular evolution of genes contained in sex chromosomes compared to their autosomal orthologs [Rice, 1984; Vicoso and Charlesworth, 2006]. The pattern varies depending on the type of mutation (new or standing, dominant or recessive), its chromosomal location (autosomal, X-linked, Y-linked), and the selection regime (i.e. whether the mutation is beneficial to one or both sexes) [reviewed in Charlesworth et al., 1987 and Mank et al., 2007]. These and other examples illustrate how the evolution of the master regulator of the gene network underlying sexual development and its mode of inheritance has profound consequences on the evolution of sexual selection, sexual dimorphism, sexual conflict, sex ratio, and ultimately, speciation and extinction [e.g. Berry and Shine, 1980; Rice, 1984; Lindholm and Breden, 2002; Fitzpatrick, 2004; Pomiankowski et al., 2004; Edwards et al., 2005; Saether et al., 2007; Vuilleumier et al., 2007].

For instance, a corollary derived from those observations is that since pure TSD taxa lack sex chromosomes [Valenzuela et al., 2003], one may predict that the number of genes with sex-specific advantage is higher in GSD than in TSD because the presence of sex chromosomes in the former favors their accumulation. Alternatively, the evolutionary rates of genes that confer a sex-specific advantage and are autosomal in TSD taxa but linked to sex chromosomes in GSD taxa should differ, because selection operates differently on genes that have intralocus conflict generated from sexual antagonisms depending on the type of chromosomes in which such genes are located [e.g. Gibson et al., 2002; Lindholm et al., 2004; Pischedda and Chippindale, 2006]. Finally, the delay in the realization of the primary sex ratio in TSD taxa and of secondary sex ratio in fish, imply that the time at which selection operates can differ between GSD and TSD taxa.

However, because the master trigger for sexual development might be linked to other sex-related traits, strong selection on these traits acting at the individual and pop-

ulation level will exert a significant effect on the evolution of the genetic regulation of sexual development as well [e.g. Wilkins, 1995; Pomiankowski et al., 2004]. Indeed, perhaps the best example of such bidirectional influence is exemplified in the evolution of environmental sex determination (ESD). Here, the potential selection for phenotypic plasticity acting at the individual and population level favors the evolution of a regulatory system of sexual development that enables the embryo to assess its environment and differentiate into the sex that will attain the maximum potential fitness given the external conditions [Charnov and Bull, 1977; Bull, 1983; Valenzuela, 2004].

A Model of the Evolutionary Transitions between GSD and TSD

In light of the similarities and differences between GSD and TSD mechanisms described here, what can be said about the repeated transitions between them? How can one make sense of the observations from developmental biology to explain such recurrent evolution? Although a definitive answer awaits further research, I propose that part of the solution to this long standing riddle lies in the differences in the timing of events between GSD and TSD previously identified. Specifically, heterochronic changes [Alberch et al., 1979] in the developmental timing of sex specification and determination, and the overlap of sex differentiation with those steps in TSD taxa (fig. 2) may confer developmental flexibility to the embryo, allowing it enough time to assess its environment before it commits to a sexual fate. As such, these heterochronic changes are adaptive because they enable a phenotypically plastic embryonic response to an unpredictable environment [Charnov and Bull, 1977; Moran, 1992]. The heterochronic changes in these developmental windows alleviate the strong selection on the GSD trigger, allowing the possibility of ESD mutations to spread.

Since overlapping developmental windows enable additional environmental feedback to the developmental process, the combination of overlapping developmental windows with an ESD 'trigger' would then be selectively favored.

Conversely, a heterochronic shift from overlapping to discrete developmental events, in a selective background of genotypic sex determination, would also be favored. In GSD, the sequential nature of the steps of specification, determination, and differentiation (fig. 2) would allow the earlier expression of sexually dimorphic traits that increase fitness [e.g. Gutierrez-Adan et al., 2006; Morton et al., 2007].

As proposed, this developmental heterochrony model is not mutually exclusive with, but rather complementary to existing hypotheses attempting to explain the evolutionary transitions between sex determining mechanisms. From the perspective of evolutionary theory, the origin, divergence, or loss of GSD and TSD, and therefore the transitions between GSD and TSD could be neutral or adaptive, and the evolutionary forces acting at each step may differ [reviewed in Valenzuela, 2004]. Such models concentrate on the level of inheritance of the master trigger of sexual development and thus, on the conditions under which the regulation of the genetic network under strict genetic control or environmental control may be favored. Other models concentrate on explaining the evolutionary transitions between GSD mechanisms as between male and female heterogamety [e.g. Charnov and Bull, 1977; Bull, 1983; Ogata et al., 2003; Vuilleumier et al., 2007], and thus, they also focus on the master trigger of sexual development. In contrast, the developmental heterochrony model focuses on changes in developmental timing primarily and the indirect changes in the initial trigger of sexual development that may have occurred as a consequence. Another explicit developmental model of the evolution of sex determination proposes that the hierarchy of its genetic pathway evolves from the bottom-up, as a series of evolutionary steps that favor the addition of neomorphic regulators upstream of the cascade each responding to frequency-dependent sex ratio selection at the population level [Wilkins, 1995]. To explain the transitional steps in this evolutionary process, Wilkins [1995] proposed that the fixation in the population of each of these novel regulators in the genetic pathway will be possible by their linkage to another gene under positive selection, or by their role in another biological function for which they are positively selected.

Under the scenario proposed here, selection operating on the developmental heterochronic changes may be stronger than the selection acting on the nature of the master trigger of the gene regulatory network that controls sexual development. In other words, this model proposes that it is the developmental shifts in the timing of events per se that are selected *for* and that allow a subsequent shift in the master trigger of sexual development [selection *of;* sensu Sober, 1984]. Alternatively, these heterochronic changes may result in a shallowing of the adaptive valleys, such that they facilitate the evolutionary transitions proposed by Wilkins' [1995] model. Further research is needed to distinguish among these hypotheses. If these heterochronic changes are truly significant, transitions between GSD and TSD mechanisms would be less restricted than previously anticipated and the lability of sex determination more easily explained.

Conclusions

Explaining the remarkable diversity of mechanisms employed by organisms to produce males and females remains an unconquered frontier in evolutionary biology. Here I discuss the significant reciprocal contributions made by evolutionary genetics and developmental biology about the function and evolution of sex determining mechanisms, particularly vertebrate GSD and TSD. By looking at these two perspectives a model and predictions are proposed about the gene regulatory network underlying sexual development and the evolutionary transitions among sex determining mechanisms. Significant differences are discussed ranging from the use of terms such as 'sex determination', to the applicability of concepts such as primary or secondary sex ratio. Most importantly, it is noted that modifications in the timing of events during development can be viewed as heterochronic changes between GSD and TSD that alleviate the strong selection on the master trigger of male and female sexual development, enabling the evolutionary transitions between genotypic and environmental sex determination. Therefore, integrating the extensive knowledge gained from evolutionary genetics and developmental biology should provide a more comprehensive view about how males and females are produced as well as why such variety evolved in the first place.

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