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Evolutionary Transitions between Sex-Determining Mechanisms: A Review of Theory

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Key Words

Genetic conflict · Maternal effects · Sex chromosomes · Sex-differential fitness effects · Sex ratio selection · Sexual antagonism

Abstract

The extraordinary diversity of sex-determining mechanisms found in nature is thought to have arisen by the addition, modification or replacement of regulators at the upstream end of the sex-determining pathway. The spread of a novel regulator of sex determination can manifest itself by an evolutionary transition between environmental and genetic sex determination, for example, or between male and female heterogamety. Both kinds of transition have occurred frequently in the course of evolution. In this paper, various evolutionary forces acting on sex-determining mutations that can bias transitions in one direction or the other are reviewed. Furthermore, the adaptive significance of the main modes of sex determination are discussed, and the common principle underlying ultimate explanations for environmental sex determination, genetic sex determination and maternal control over sex determination in the offspring are highlighted. Most of the current theory concentrates on the population-genetic aspects of sex determination transitions, using models that do not reflect the developmental mechanisms involved in sex determination. However, the increasing availability of molecular data creates opportunities for the future development of mechanistic models that will further clarify how selection and developmental architecture interact to direct the evolution of sex determination genes.

Biparental reproduction, involving 2 individuals of distinct sexes, is the dominant mode of reproduction in many animal taxa, including arthropods, nematodes and vertebrates [Bull, 1983; Barnes et al., 2001]. Its evolutionary origin in animals presumably dates back more than 550 mya, to the last common ancestor of the coelomate bilaterians [Haag and Doty, 2005]. Biparental reproduction is also found in other groups of sexually reproducing organisms, such as dioecious algae and flowering plants [Ainsworth, 2000; Umen, 2011], as well as heterothallic fungi [Heitman et al., 2013]. In this diverse group of species, development passes through a critical stage at which the zygote commits irreversibly to either the male or the female sexual fate, resulting in a separation of the sexes in different individuals [Valenzuela, 2008]. This ontogenetic process, known as sex determination, triggers the dif-

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ferentiation of specialized male or female reproductive organs and organizes sex-specific differences in gene expression, physiology, morphology, and behavior (sex differentiation) [Badyaev, 2002; Ellegren and Parsch, 2007].

Given the far-reaching consequences of sex determination for the life history of the individual [Andersson, 1994] and its immediate effect on the population sex ratio [Charnov, 1982], one would expect sex-determining mechanisms to be subject to strong selection. In fact, when individuals differ in their genetic background or experience different environmental conditions during development and not all components of such variation affect males and females equally [e.g. Chippindale et al., 2001; Warner and Shine, 2008], it is not optimal to assign sex randomly, i.e. independently of these differences [Leimar, 2005]. Instead, selection favors a conditional sex determination strategy that adaptively switches development into the male or female trajectory contingent on some genetic or environmental cues [Charnov and Bull, 1977; Rice, 1986; Van Dooren and Leimar, 2003].

Which particular cue is used for sex determination in a particular species is predicted to depend on the sources of variation to which individuals are exposed during development and to what extent these have differential fitness effects on the sexes. This may often be determined by such factors as the ecology and life history of the species [Pen et al., 2010], its genetic architecture [Leimar et al., 2004; Uller and Helanterä, 2011] and other characteristics that are likely to vary across species. From an adaptationist perspective, it is therefore not surprising that sex-determining mechanisms are found to be highly diverse across taxa, with some species using a specific environmental cue (e.g. temperature, photoperiod or population density) as the primary signal in sex determination (environmental sex determination; ESD) and others relying on various types of genetic sex determination (GSD) such as male or female heterogamety, haplodiploidy or multilocus sex-determining mechanisms [Bull, 1983; Marshall Graves, 2008; Janousek and Mrackova, 2010]. Consistent with this idea, several taxa show evidence of frequent evolutionary transitions between different modes of sex determination [Hillis and Green, 1990; Kraak and Pen, 2002; Ezaz et al., 2006; Mank et al., 2006].

Despite the diversity of primary sex-determining signals, downstream sex determination and sex differentiation genes are conserved in function across species and potentially across phyla [reviewed in Pask and Marshall Graves, 1999; Zarkower, 2001; Hodgkin, 2002; Haag and Doty, 2005; Shukla and Nagaraju, 2010]. This observation is in line with the notion that sex determination, like other processes acting in mid-development, is subject to strong pleiotropic constraints [Marín and Baker, 1998; Kalinka and Tomancak, 2012]. Moreover, molecular genetic studies in model organisms and their close relatives indicate that downstream genes in the sex determination pathway are more highly conserved than upstream ones [Zarkower, 2001]. This striking pattern, observed for the well-studied sex determination pathways of *Drosophila melanogaster* [Nöthiger and Steinmann-Zwicky, 1985; Gempe and Beye, 2011], *Caenorhabditis elegans* [Zarkower, 2001] and vertebrates [Marshall Graves, 2008; Mawaribuchi et al., 2012], is widely regarded as support for the hypothesis that sex determination pathways evolve from the bottom up, by the successive addition of novel upstream regulators [Nöthiger and Steinmann-Zwicky, 1985; Wilkins, 1995; Marín and Baker, 1998].

This review focuses on the evolutionary forces that act on such novel regulators when they first appear in a population and on the adaptive aspects of transitions between different modes of sex determination [see also Beukeboom and Perrin, in press]. The first theoretical studies in this area were published by Bull and Charnov [1977] and Bull [1981], well before the molecular structure of sex determination pathways was elucidated. As a consequence, these classical studies, as well as many later theoretical models building on them, are not explicit about the developmental mechanisms involved in sex determination, nor are they framed in terms of a specific hypothesis for the mode of pathway evolution. The theoretical literature, with its focus on the spread of hypothetical sex determination modifiers, can therefore be criticized as being too abstract [Pomiankowski et al., 2004; Uller and Helanterä, 2011]. On the other hand, by bypassing the complexity of development, the models have been able to explore systematically the intricate population genetics of sex determination transitions, generating clear insights into the diverse evolutionary forces that act on sex determination genes. The challenge for the future is to integrate these insights in the current knowledge of the molecular mechanisms, in order to clarify how selection and developmental architecture interact in the evolution of sex determination.

Neutral Evolution

One fundamental insight offered by population-genetic models is that evolutionary transitions between sex-determining mechanisms can occur via selectively neutral intermediate states in which multiple polymorphic

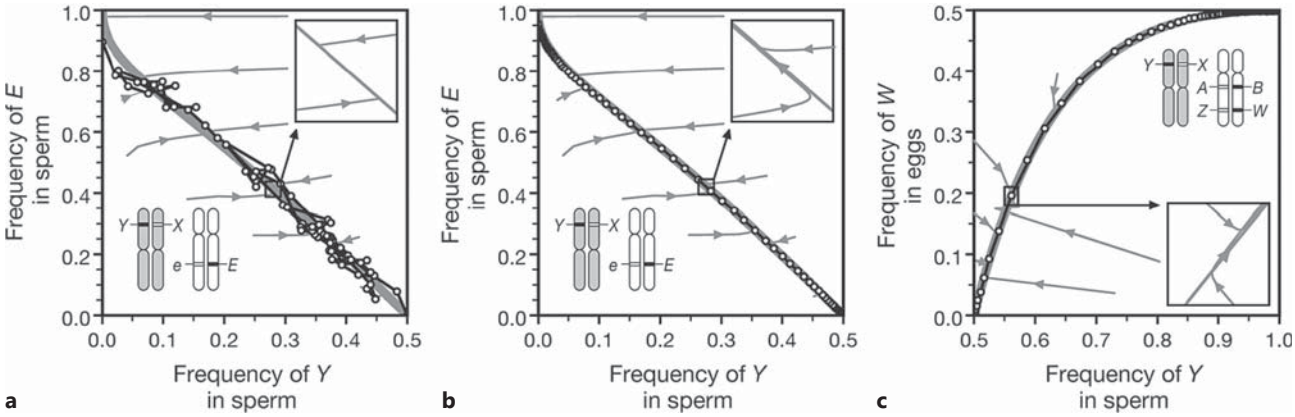

Fig. 1. Neutral and adaptive models of sex determination transitions. The 3 panels illustrate the change of the gamete frequencies of ancestral (horizontal axes) and novel (vertical axes) sex-determining alleles during a transition from an ancestral XX♀/XY♂ genetic sex determination system to either ESD (**a**, **b**) or ZZ♂/ZW♀ GSD (**c**) (female heterogamety). For each panel, genetic assumptions are illustrated by means of schematic representations of the ancestral sex chromosome pair (grey) with the sex-determining locus and a pair of autosomes (white) carrying a sex determination mutation (**a–c**) and a sex-antagonistic locus (**c**). **a** Invasion of an autosomal mutation E that induces XX individuals to develop as males under some environmental conditions (comparable to the scenario analyzed by Bull [1981]). In the absence of intrinsic fitness differences between sex determination genotypes, the allele E can drift to fixation as the population moves stochastically along a line of equilibria (thick grey line), away from the ancestral state in the lower right corner of the diagram. Once variation at the ancestral sex-determining locus is lost and the allele E has gone to fixation (in the upper left corner of genotype space), sex is entirely determined by environmental cues. Populations initialized with arbitrary combinations of allele frequencies quickly evolve towards the line of equilibria (thin grey trajectories), under the influence of selection for a balanced sex ratio. The process of drift along the line of equilibria is illustrated by the results from a stochastic, individual-based implementation of the population-genetic model (black trajectory with open circles; gamete frequencies are plotted every 50 generations for a population of 1.000 individuals). **b** Model assumptions are as in **a**, except that the environment now has sex-differential effects on fitness, and the allele E reverses the sex of XX zygotes predominantly in those environments where it is beneficial to develop as a male. As a result, the invasion of E is favored by selection, indicated by the slow, deterministic change of allele frequencies along a path close to the former line of equilibria (results are plotted every 50 generations for a large population of 1×10^6 individuals). The inset shows a close-up of 2 trajectories, illustrating how the line of equilibria has changed into a nearly neutral path (compare with the inset in panel **a**). Qualitatively similar dynamics are observed for other types of transitions between sex-determining systems, including transitions between male and female heterogamety (**c**). Shown is the invasion of an epistatically dominant feminizing mutation W that is located on an autosome in close proximity to a sexually antagonistic locus. The spread of the novel sex-determining allele is driven by indirect selection, supported by the development of linkage disequilibrium between W and the female beneficial allele B [van Doorn and Kirkpatrick, 2007, 2010]. The evolutionary trajectories follow a nearly neutral path that is close to the line of equilibria (thick grey line) calculated for a model without sex-antagonistic selection [Bull and Charnov, 1977].

sex determination loci determine sex [Scudo, 1967]. For example, Bull [1981] considered a population with XX♀/XY♂ GSD (male heterogamety) as the ancestral state, in which some XX individuals developed as males in extreme environments. The range of environmental conditions inducing sex reversal was assumed to be under control of a quantitative genetic trait, while the presence of variation in this trait allowed the level of ESD to evolve. Bull showed that whenever the level of ESD changed, the frequency of XY changed as well to restore an equal sex ratio. He also demonstrated that there is no tendency for selection to change the level of ESD once the sex ratio has equilibrated, unless some sex determination genotypes are assumed to be intrinsically more fit than others. In the absence of other selective forces, it is thus conceivable that demographic stochasticity or other sources of random variation induce a transition to ESD by causing the population to drift through genotype space along a line of equilibria connecting the alternative modes of sex determination (fig. 1a). A similar continuous path of neutral equilibria between different modes of sex determination has been observed in population-genetic analyses of mul-

tifactorial GSD [Scudo, 1967], one-locus multi-allele sex determination [Karlin and Lessard, 1984] and in models of transitions between male and female heterogamety (ZW♀/ZZ♂ GSD) (fig. 1c) [Bull and Charnov, 1977].

The existence of a line of equilibria in models without intrinsic fitness differences has several biological implications. First, generic sex-determining mechanisms, like ESD or male and female heterogamety, do not necessarily represent different peaks in the adaptive landscape, so populations in transition from one mechanism to another do not necessarily have to pass through a fitness valley [compare Valenzuela, 2008]. Second, only small fitness differences between genotypes suffice to induce a consistent movement of the population along the line of equilibria (fig. 1b, c) towards one of the alternative single-factor sex-determining mechanisms [Bull and Charnov, 1977; Bull, 1981]. In practice, sex determination transitions may therefore be driven by subtle sources of selection, such as those acting indirectly via linkage disequilibria with other loci [van Doorn and Kirkpatrick, 2007], or by a bias generated by the interaction of sex ratio selection and demographic stochasticity around the line of equilibria [Vuilleumier et al., 2007].

Following the same argument, the notion of neutral transitions between sex-determining mechanisms is biologically relevant mainly as a null model or first approximation of the evolutionary dynamics. In fact, any source of additional selection, however weak, acting on the sex determination genes transforms the line of equilibria into a nearly neutral path along which populations will evolve deterministically in one direction or the other (fig. 1b, c). This may be one reason why multifactorial sex determination is not often found in nature [Bull, 1983]. Rare examples among species with genetic sex determination include the housefly *Musca domestica* [Kozielska et al., 2006] and the platyfish *Xiphophorus maculatus* [Orzack et al., 1980]. In addition, a few intermediate forms between ESD and GSD with major gene effects exist [e.g. Girondot et al., 1994; Shine et al., 2002], whereas quantitative genetic variation in sex determination thresholds in species with ESD is not uncommon [McGaugh and Janzen, 2011]. In all of these cases, rather than representing a temporary transitional state, multifactorial sex determination appears to be maintained as a consequence of specific relative fitness differences between genotypes [Orzack et al., 1980; Girondot et al., 1994; Feldmeyer et al., 2008]. Therefore, both the rarity of multifactorial sex determination and its apparent maintenance by selection in species with unusual sex determination provide an argument for exploring the role of adaptive mechanisms in sex determination transitions.

Below, 2 classes of adaptive mechanisms are discussed. The first class encompasses sex ratio selection and pleiotropic fitness benefits, which can both drive the spread of a novel sex-determining mutation by means of direct selection. This type of selection results from fitness differences between alternative alleles at the sex-determining locus that exist independently of the genetic background or environmental conditions. Alternatively, fitness differences can be generated indirectly, by means of an interaction between sex determination and environmental, genetic or epigenetic sources of variation. The corresponding indirect selection mechanisms will be reviewed in the section 'sex-by-environment, sex-by-genotype and sex-by-parental-condition interaction effects on fitness'.

Sources of Direct Selection on Novel Sex-Determining Genes

Sex Ratio Selection

Given the intricate connection between sex determination and the sex ratio, deviations of the sex ratio from its optimum generate strong selection on sex determination genes. A sex determination transition is a likely response to sex ratio selection if there is genetic variation for an alternative sex-determining mechanism capable of producing a sex ratio closer to the optimum. Bulmer and Bull [1982] were the first to point out that this mechanism could lead to transitions from ESD to GSD, for instance, by the accumulation of genetic variation at loci affecting the reaction norm for ESD. Their argument builds on the idea that spatial or temporal variation in environmental conditions can cause fluctuations in the population sex ratio away from its usual 1:1 optimum. If the cost of producing a suboptimal sex ratio outweighs the fitness benefit of using ESD (i.e. being able to adaptively match offspring sex to the state of the environment; see below), pure ESD, using a switch-like threshold reaction norm, is no longer evolutionarily stable.

One potential evolutionary response is to resort to partially random sex determination. This can be accomplished by introducing developmental noise in the mapping from environmental conditions to offspring sex, which reduces the slope of the reaction norm for ESD at its switching point (fig. 2a) [Van Dooren and Leimar, 2003]. Alternatively, genetic variation may accumulate at loci that control the location of the threshold of the ESD reaction norm (fig. 2b). Selection for increased genetic variance at these loci supports the invasion of a gene with major effect on sex determination, marking the transition

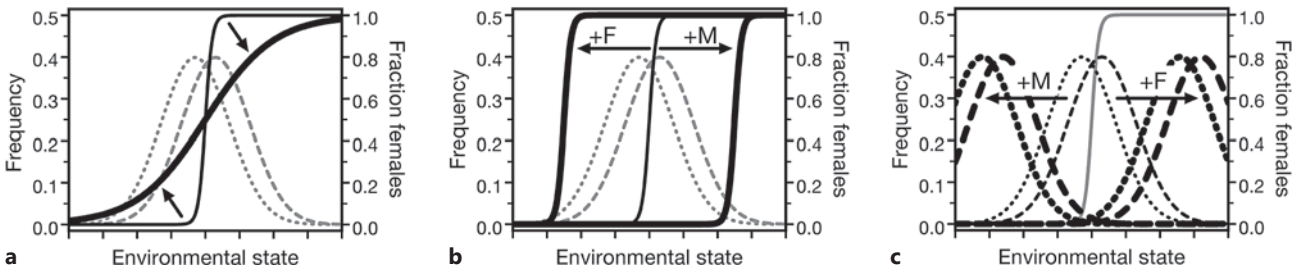

Fig. 2. Three different mechanisms for the transition from ESD to GSD. Spatial or temporal variation in environmental conditions (as an example, dashed and dotted curves symbolize year-to-year fluctuations in the distribution of environmental states) can lead to a maladapted, biased population sex ratio in species with ESD [Bulmer and Bull, 1982]. **a** One potential evolutionary response to alleviate this problem is to resort to partially random sex determination by introducing developmental noise in the mapping from environmental conditions to offspring sex [Van Dooren and Leimar, 2003]. The introduction of developmental noise causes a reduction of the slope of the reaction norm at its inflection point (original and adjusted reaction norms are indicated, respectively, by thin and thick black curves). **b** A second scenario is that loci controlling the location of the threshold of the reaction norm become polymorphic, such that some genotypes develop as male under a broad range of environmental conditions (arrow +M), whereas other genotypes develop predominantly as females (arrow +F). In this way, a transition from ESD to GSD can be realized by the invasion of a gene with major effect on sex determination [Bulmer and Bull, 1982]. **c** A third possibility, originally suggested by Kraak and de Looze [1993] in the context of genetic modifiers of embryonic growth rate, is that genetic variation substitutes for the proximate cue produced by variation in environmental conditions, such that some genotypes consistently produce males (arrow +M) while others consistently produce females (arrow +F).

to GSD [Bulmer and Bull, 1982; Van Dooren and Leimar, 2003]. Both solutions effectively dampen sex ratio fluctuations at the cost of simultaneously reducing the fitness benefits associated with ESD. However, GSD often has fitness benefits of its own, which would favor the ESD-GSD transition (fig. 2b) over the transition to partially randomized sex determination (fig. 2a). Whether this bias is relevant in practice depends on how much heritable variation is available for each alternative evolutionary outcome.

Although species with ESD are more prone to exhibit suboptimal sex ratio fluctuations, they might enjoy a fitness advantage in situations where selection favors a biased sex ratio, for example, due to local mate competition or different energetic costs to the parents for the production of sons versus daughters [Charnov, 1982]. According to this argument, ESD is more flexible and permits faster evolution towards an optimal biased sex ratio than common GSD mechanisms, which are constrained by the segregation ratio in the heterogametic sex [Bull, 1983; Charnov and Bull, 1989; Freedberg and Wade, 2001]. However, empirical studies in species with ESD offer no support for the hypothesis that selection for a biased sex ratio has been a key driving factor in the origin or maintenance of ESD [Shine, 1999; Janzen and Phillips, 2006].

Several lines of evidence suggest that GSD does indeed offer little scope for adjusting the sex ratio, but this seems to be only a mild evolutionary constraint for species with GSD. Among species with seemingly inflexible GSD (i.e. diplo-diploid species with sex chromosomes), there is overall little evidence of modifications to the sex-determining mechanism in response to sex ratio selection [Krackow, 2002]. Several explanations have been offered for this observation. First, species with male or female heterogamety show almost no heritable genetic variation for sex ratio [Bulmer and Bull, 1982; Bull and Charnov, 1988]. Potential mechanisms for sex ratio control other than sex determination (e.g. sperm selection or selective resorption of embryos) may therefore be more likely to evolve in order to accommodate a biased sex ratio. In addition, it may be difficult to adapt an established male- or female-heterogametic sex-determining system into a flexible mechanism capable of producing the desired biased sex ratio because genetic modifiers of the ancestral mechanism may offer limited opportunities for sex ratio control [Uller et al., 2007]. For example, Kozielska et al. [2006], using a model inspired by the genetics of sex determination in the housefly, showed that selection for a biased sex ratio could maintain genetic polymorphism at multiple sex-determining loci. However, the sex ratio bias

produced by the resulting multifactorial sex-determining mechanism was much smaller than the value predicted by sex allocation theory, due to population-genetic constraints.

A similar point was made by Werren et al. [2002], who studied the evolution of sex-determining genes in zygotes in response to maternally induced manipulation of the sex ratio. One scenario considered in their analysis is when the genes of the zygote favor a female-biased sex ratio that is less strongly skewed than the optimal sex ratio from the mother's perspective. While mother and offspring are in evolutionary conflict, selection favors the expression in the zygote of a dominant masculinizing gene that pushes the sex ratio in the direction of the zygote's optimum. However, as the arms race between maternally and zygotically expressed genes leads to the establishment of male heterogamety, the sex-determining system ultimately loses the capacity to produce a female-biased sex ratio.

Conflict between parents and offspring over the sex ratio is common [Uller et al., 2007], and offspring often favor a less biased sex ratio than their parents. The predictions of standard sex ratio theory, which generally assumes parental control, might therefore be misleading in species with sex chromosomes, where parental genes are much more limited in their options to interfere with sex determination than genes expressed in the zygote. One would therefore expect less biased sex ratios in these species than would be predicted by sex ratio theory, which may be another important reason for the apparent lack of adaptive sex ratio adjustment in higher vertebrates [Krackow, 2002].

Even when selection favors a balanced sex ratio and the sex-determining mechanism is capable of producing equal numbers of males and females, population sex ratios may still deviate markedly from 1:1, due to the manipulation of sex determination by selfish genetic elements. The resulting distortion of the sex ratio generates sustained sex ratio selection that may evoke counter-adaptations of the sex-determining system [Werren and Beukeboom, 1998]. For example, in the Spanish mole, *Talpa occidentalis* [McVean and Hurst, 1996], the creeping vole, *Microtus oregoni* [Charlesworth and Dempsey, 2001], and some other rodent species, genetic conflict between sex-linked segregation distorters and their modifiers seems to have driven the evolution of unusual sex chromosome systems. Genetic conflicts can also drive transitions between male and female heterogamety, as shown by various models. For example, under sex chromosome meiotic drive [Jaenike, 2001], sex ratio selection favors the invasion of a novel sex-determining allele that produces individuals of the underrepresented sex [Kozielska et al., 2010]. As the new sex-determining locus spreads, polymorphism at the ancestral locus is lost, eventually leading to the demise of the sex ratio distorter. Similarly, the repression of cytoplasmic sex ratio distorters by host masculinizing alleles can induce a switch from female heterogamety to a multifactorial system dominated by male heterogamety [Caubet et al., 2000].

Pleiotropic Benefits

If a novel sex-determining mutation confers a higher viability or another intrinsic fitness advantage to its carrier, the mutation can spread as the result of simple natural selection. Bull and Charnov [1977] observed that models incorporating such direct selection behaved similar to their equal fitness counterparts in that the genotype frequencies initially approached a region near the line of equilibria of the corresponding equal fitness model. In that region of genotype space, the invading sex-determining mutant is unaffected by sex ratio selection, but still experiences positive natural selection. The frequency of the mutant therefore increases while the genotype frequencies continue to follow approximately the neutral line of equilibria, until the genetic polymorphism at the ancestral sex-determining locus is lost.

A serious problem for the pleiotropic benefit hypothesis of sex determination transitions, already noted by Bull and Charnov [1977], is that there are few obvious biological reasons why a novel sex-determining allele would have an intrinsic fitness advantage over the ancestral ones. One realistic possibility that is nearly indistinguishable from pleiotropy in practice is that the invading sex determiner is in strong positive linkage disequilibrium with an allele that is favored by selection [Bull, 1983]. For example, some sex determination variants in the housefly appear to be linked to pesticide resistance alleles [Werren and Beukeboom, 1998]. As the beneficial allele sweeps to fixation, it drags along the genetically associated sex-determining allele, giving it the appearance of being favored by selection itself [Barton, 2000]. However, such 'genetic hitchhiking' proceeds for as long as there is genetic variation at the locus under selection, and recombination has not broken down the linkage equilibrium. Accordingly, hitchhiking can only facilitate a sex determination transition if the sex determiner is tightly physically linked to a polymorphic locus under strong positive selection. This is quite unlikely, except perhaps when both the sex-determining mutation and the novel beneficial allele arise on a chromosomal inversion [Kirkpatrick, 2010].

The alternative possibility that the novel sex determiner itself is favored by direct selection has sometimes been considered in theoretical studies, but is then usually discarded on the basis of parsimony because it requires a fortuitous type of pleiotropy [e.g. Rice, 1986]. A notable exception is provided by Kraak and de Looze [1993], who proposed a verbal model for the evolution of sex determination in vertebrates, in which a gene under selection gains control over sex determination. Their hypothesis builds on the assumption that the growth rate of the undifferentiated gonads in the developing embryo acts as a proximate cue for sex determination in species with ESD. The association between offspring sex and developmental growth rate may have evolved because individuals of one sex benefit more from developing fast than individuals of the other sex. Kraak and de Looze [1993] then supposed that there is genetic variation that affects growth rate in the same way as variation in the environment does. A gene causing the undifferentiated gonads to develop more rapidly would then be able to substitute for the role of the environment and act as a trigger for sex determination. Furthermore, due to previous adaptation of the ancestral ESD mechanism, such a gene would always be expressed in the sex in which its effect on embryonic growth rate is beneficial, giving it a significant fitness advantage. Note that the hypothesis of Kraak and de Looze [1993] shows important similarities with Bull's analysis of transitions between ESD and GSD [Bull, 1981] discussed earlier, with the crucial difference that not the threshold but the input variable (including its fitness-relevant aspects) of the reaction norm for ESD is assumed to be under genetic control (fig. 2c).

A formal analysis, using individual-based computer simulations, shows that transitions from adaptive ESD to GSD as proposed by Kraak and de Looze [1993] are feasible if genetic variation for growth rate is concentrated at a single locus [Kraak et al., 2000]. However, when genetic variation can accumulate at multiple loci, growth rate-accelerating alleles become genetically associated with each other only when size benefits differ maximally between males and females, and several additional restrictive conditions are met [Kraak and Pen, 2002]. Therefore, without empirical evidence for major effect genes that modify sex determination and that also differentially affect the fitness of males and females by their impact on embryonic growth rate (or any other fitness-relevant process involved in sex determination), the role for pleiotropic benefits in triggering sex determination transitions appears rather limited. This conclusion does not rule out the possibility that pleiotropic fitness effects are important to stabilize an established sex determination system. For example, the *Sxl* gene in *Drosophila* regulates both *tra*, its downstream gene in the sex determination cascade, and *msl-2*, a gene involved in dosage compensation. Due to the latter interaction, which became critical after the establishment of *Sxl* and the differentiation of its sex chromosome [Pomiankowski et al., 2004], replacement of *Sxl* by another sex-determining switch is likely to have severe detrimental effects.

Sex-by-Environment, Sex-by-Genotype and Sex-by-Parental-Condition Interaction Effects on Fitness

After reviewing sources of direct selection, this paper continues with indirect selection mechanisms, which rely on an interaction between sex determination and another source of variation with sex-differential fitness effects. Three such sources of variation have been discussed in the literature: environmental variation, sexually antagonistic genetic variation and variation in parental condition with transgenerational effects.

Environmental Variation with Differential Fitness Effects on the Sexes

Environmental sex determination has been extensively studied in fish and amniote vertebrates, where it occurs predominantly as temperature-dependent sex determination (TSD). Low temperatures tend to delay embryonic development in ectotherm animals, resulting in delayed birth or hatching with likely fitness consequences later in life due to a smaller size at reproduction [Shine, 1999; Janzen and Phillips, 2006; Warner and Shine, 2008; Pen et al., 2010]. Males and females usually differ in how their fitness is affected by variation in temperature during development. In some species, females benefit more than males from having a large size at reproduction because the reproductive output of a female increases more steeply with body size than that of a male [Conover, 1984]. In other species, having a large body size is particularly important to males because their fitness depends on their relative size advantage in male-male contests [Andersson, 1994]. In general, therefore, the ratio of male to female fitness varies with environmental conditions.

Charnov and Bull [1977] were the first to point out that when a zygote finds itself in an environment where its fitness would be higher if it were to develop as a male rather than as a female, then selection would favor it becoming a male. In other words, a sex determination strategy that matches offspring sex to the one that does relatively

better in a particular environment will outperform random sex determination. This argument is similar to the ultimate explanation of conditional sex allocation strategies in species where parents exert control over the sex ratio of their offspring in response to their own condition [Trivers and Willard, 1973]. It also closely resembles adaptive theories predicting the timing of sex change in sequential hermaphrodites [Warner et al., 1975], which tend to spend the first part of their life in the sex where the relative disadvantage of being young or small is least.

The Charnov-Bull model is currently the best-supported hypothesis for the evolution of ESD [Shine, 1999]. Nevertheless, the empirical evidence is not unambiguous, particularly not among reptiles, in which ESD is found relatively often [Janzen and Paukstis, 1991]. One reason for this may be that ESD is phylogenetically ancient in crocodiles and turtles, which have been the main subject of empirical studies on TSD. The current relationship between offspring sex and incubation temperature in these groups may therefore no longer reflect the adaptive value of TSD at the time when it originated [Janzen and Phillips, 2006]. On the other hand, studies in lizards, targeted at species in which TSD has evolved recently, have documented convincing support for the Charnov-Bull model [Warner and Shine, 2008].

Genetic Variation in Traits that Differentially Favor Males and Females

Genetic variation segregating in a population can affect males and females differentially, in the same way as environmental variation often does. In fact, many traits that are expressed in the context of sexual selection and reproduction have sex-specific optima, due to the functional divergence of male and female gender roles [Parker, 1979]. Because males and females share a common gene pool, the genetic conflict between the sexes over the expression of these traits (also known as intralocus sexual conflict [van Doorn, 2009]) cannot always be resolved by the evolution of sexual dimorphism.

Intralocus sexual conflict may result in the maintenance of sexually antagonistic genetic variation in a population [Brommer et al., 2007; Foerster et al., 2007], with some individuals carrying alleles that are beneficial in males but deleterious in females and others carrying alleles with the opposite fitness effects. In close analogy to Bull and Charnov's argument for ESD, one would thus expect selection to favor a conditional sex determination strategy that would adaptively match the sex of an individual to its genetic background. That means that zygotes should develop as males when they carry sex-antagonistic alleles enhancing male fitness, whereas zygotes carrying female-beneficial alleles should develop as females. One way for evolution to realize such a strategy is to have a sex determination gene within a short distance of sexually antagonistic genes on the chromosome (fig. 1c). Tight physical linkage is associated with a low rate of recombination, which enables selection to maintain strong linkage disequilibrium between the sex determiner and sexually antagonistic genes.

In line with this argument, Rice [1986] demonstrated that a sexually antagonistic gene, tightly linked to a dominant masculinizing mutation, facilitated the spread of the mutant sex-determining allele in a population with polygenic sex determination as ancestral state. The transition from polygenic to single-locus GSD was critically supported by the linkage disequilibrium between the masculinizing mutation and the sex-antagonistic gene. Due to the genetic correlation between alleles at the 2 loci, individuals that inherited the mutation were more likely to inherit the male-beneficial allele as well. This gave them a selective advantage over other types of males, allowing the major sex-determining gene to spread, eventually resulting in the loss of polygenic sex determination.

The same indirect selection mechanism can cause transitions between different single-locus genetic sex-determining systems, resulting in the establishment of a new sex-determining locus, the evolution of a new sex chromosome pair or a transition between male and female heterogamety. The evolutionary dynamic of such GSD transitions is complicated by the fact that also the ancestral sex-determining locus might be linked to sexually antagonistic genes. Van Doorn and Kirkpatrick [2007] therefore analyzed the relatively simple scenario of a novel masculinizing mutation arising on an autosome, in a population with male heterogamety. In their model, both sex-determining alleles were assumed to be linked to a sexually antagonistic gene. The autosomal sex-antagonistic gene favored the invasion of the masculinizing mutation, in the same way as discussed by Rice [1986]. However, sexually antagonistic variation segregating on the ancestral sex chromosomes inhibited the spread of the mutant because males carrying the novel masculinizing allele also carried 2 X chromosomes enriched for female-beneficial alleles. Mutant males were therefore more likely to inherit the autosomal male-beneficial allele, but also more likely to inherit the female-beneficial allele on the ancestral sex chromosomes than males carrying an ancestral Y chromosome.

Depending on the net fitness effect of these 2 indirect selection forces, van Doorn and Kirkpatrick [2007] iden-

tified 2 generic evolutionary outcomes: either the masculinizing mutation was lost, or it spread and replaced the ancestral sex-determining gene. The latter outcome, which led to the loss of the ancestral Y chromosome and the establishment of a new sex chromosome pair, occurred when the autosomal sex-antagonistic locus harbored more genetic variation, when its alleles had stronger sex-antagonistic fitness effects or when it was more tightly linked to its nearby sex-determining gene than the sexually antagonistic gene on the ancestral sex chromosome. In addition, tight linkage allowed for evolutionary bistability or the maintenance of a sex chromosome polymorphism under a limited range of conditions.

The main conclusions for the scenario of Y chromosome transitions were later shown to carry over to more complicated transitions between genetic sex-determining mechanisms involving an arbitrary number of sex-antagonistic loci [van Doorn and Kirkpatrick, 2010]. This applies, for example, to the invasion of a novel sex-determining allele at the ancestral sex-determining locus, the establishment of a novel sex-determining gene located on the ancestral sex chromosomes and transitions between male and female heterogamety in the presence of recessive deleterious alleles on the ancestral Y chromosome. Simulations with a large number of sex-antagonistic loci suggested that the few sex-antagonistic genes that are most tightly linked to a sex-determining gene determine the direction of sex determination transitions. This would imply that the sexually antagonistic variation effectively responsible for sex determination transitions segregates at a small subset of the sex-antagonistic loci. Therefore, even a small inversion that captures both a sex-determining mutation and a single fitness-relevant sex-antagonistic locus on an autosome could conceivably hijack sex determination from the ancestral sex chromosomes.

Consistent with this suggestion, the recently evolved Y chromosome in the three-spined stickleback has experienced an inversion encompassing the sex-determining region [Ross and Peichel, 2008; Kirkpatrick, 2010]. Whether this inversion triggered the sex chromosome shift or occurred after the sex-determining locus was established (as suggested by the traditional theory for sex chromosome evolution [Rice, 1987; Charlesworth et al., 2005]) can, in principle, be tested by analyzing the patterns of neutral variation on the inversion and the neo-sex chromosome [Kirkpatrick et al., 2010; Guerrero et al., 2012a]. Further empirical support for a role of sexual antagonism in sex determination transitions comes from a growing number of cases of recently derived sex chromosomes that carry sexually selected loci [Kallman, 1970; Wada et al., 1998; Lindholm and Breden, 2002; Fernandez and Morris, 2008; Kitano et al., 2009]. A particularly interesting example is provided by a mutation in the *pax7* gene in Lake Malawi cichlid fish, which causes an orange-blotched phenotype subject to sexually antagonistic selection. This mutation originated once in Lake Malawi cichlids and appears to have spread in close association with a feminizing allele that is epistatically dominant over the ancestral XX♀/XY♂ sex-determining system [Roberts et al., 2009].

Epigenetic/Transgenerational Effects

A third source of variation with potential sex-differential fitness effects is variation in parental condition (e.g. nutritional state, social rank or stress level). Lasting effects of parental condition on offspring fitness can arise in different ways, for example, through condition-dependent parental provisioning, the inheritance of high-quality breeding sites or social rank or early-life exposure to maternal stress hormones. If sons and daughters are not affected in the same way by the transgenerational effects of variation in parental condition, selection favors conditional sex allocation by the parents over random sex allocation [Trivers and Willard, 1973]. The least costly way for the parents (typically, the mother) to exert control over the sex ratio of offspring is to interfere directly with sex determination.

In oviparous species with TSD, mothers can influence the sex of their offspring by selecting nest sites based on ground temperature or other thermally related microhabitat qualities. Heritable variation in individual preferences for nest sites with particular thermal properties has been documented in some species [Janzen and Phillips, 2006]. Moreover, nest site preferences might be culturally inherited, which would allow for a rapid adjustment of the sex ratio in response to environmental fluctuations [Freedberg and Wade, 2001]. In the snow skink *(Niveoscincus ocellatus)* and the yellow-bellied water skink *(Eulamprus heatwolei)*, 2 rare examples of viviparous lizards with TSD, pregnant females can control the sex of their offspring as well as their phenotypic traits by adjusting their thermoregulatory basking behavior [Wapstra et al., 2004; Langkilde and Shine, 2005].

Maternal effects in species with GSD are often mediated by maternal gene products (mRNA or proteins) that are placed in the developing egg. An influence of maternal gene products on sex determination has been demonstrated in various organisms [Werren et al., 2002]. For example, sex determination in the haplodiploid wasp *Nasonia vitripennis* relies on a maternal supply of *N. vitri-*

pennis transformer (Nvtra) mRNA to the egg. In fertilized eggs, the maternal mRNA switches on an autoregulatory feedback loop of zygotic *Nvtra* expression that is essential for female development [Verhulst et al., 2010]. Additionally, transcription of zygotic *Nvtra* is prevented in unfertilized eggs by genetic imprinting of the maternally inherited copy of the *Nvtra* gene [Van de Zande and Verhulst, this issue].

A Unifying Adaptive Theory?

The similarity between the ultimate explanations for the various types of conditional sex determination reviewed in this section suggests that the evolution of conditional sex-determining mechanisms can be explained by a single evolutionary principle: if there is variation in some factors with sex-specific fitness effects that can be observed (or predicted) at the time of sex determination, then selection favors a sex-determining mechanism that makes use of the available information by matching the sex of the individual to whichever sex does relatively better under the observed conditions. In other words, ESD, GSD and parental control over sex determination are favored over random sex determination when males and females are affected differentially by variation in environment, sexually antagonistic genetic variation and variation in parental condition, respectively.

More often than not, several of these sources of variation are present at the same time. For example, male and female fitness may be differentially affected by multiple, independent environmental factors, as well as sexually antagonistic genes segregating with different linkage groups. In such cases, one would expect the optimal sex-determining mechanism to respond to the single factor that is most closely correlated with fitness [Leimar, 2005], in analogy with the conditions for sex chromosome transitions under the influence of autosomal and sex-linked sexually antagonistic genes [van Doorn and Kirkpatrick, 2007, 2010]. However, this suggestion remains speculative because virtually all models of transitions between qualitatively different types of sex determination consider only a single source of sex-differential fitness variation.

Genetic and Developmental Architecture

As the structure of sex-determining pathways is being elucidated in a growing number of species, it becomes increasingly clear that details of the genetic and developmental architecture of a species shape and channel the selection pressures acting on its sex-determining genes. Understanding the interplay between adaptation and developmental mechanisms is a key challenge for future theoretical and empirical work on sex determination.

One obvious question to ask in this context is why sex determination appears to be labile in some taxa, but stable in others. For example, the evolutionary stability of sex determination in birds and mammals contrasts sharply with the high rate of transitions observed in some clades of cold-blooded vertebrates [Marín and Baker, 1998; Ezaz et al., 2006]. It could be that the evolution of heteromorphic sex chromosomes constrains future sex chromosome transitions, due to the accumulation of sexually antagonistic genes on the sex chromosomes, the reduction of recombination rates in the vicinity of the master sex-determining gene, the loss of functional genes from the Y chromosome, the evolution of dosage compensation, and the translocation of genes essential for male fitness to the Y chromosome [Bull, 1983; Rice, 1987; Charlesworth et al., 2005]. Once the sex chromosomes have become heteromorphic, XX males, XY females or YY individuals of either sex, which arise when sex determination is hijacked by another chromosome pair, are likely to suffer from reduced fertility and/or viability, inhibiting the spread of new sex-determining genes [Bull and Charnov, 1985; van Doorn and Kirkpatrick, 2007; Mawaribuchi et al., 2012].

Sex chromosome heteromorphism can explain why sex determination appears to be locked into its current state in birds and mammals. Yet, the argument so far leaves unaddressed why sex determination should continue to be labile in taxa lacking heteromorphic sex chromosomes. The lower vertebrates, which generally have undifferentiated sex chromosomes, show evidence of frequent sex chromosome transitions. For example, a phylogenetic analysis of genetic sex determination in teleost fishes found that 8 of 26 families include both species with XY and species with ZW sex determination [Mank et al., 2006], and within the subfamily *Salmoninae* (including char, trout and salmon), at least 4 different chromosomes determine sex in different species [Woram et al., 2003]. A further example of frequent turnovers between male and female heterogamety is provided by a survey in amphibians, which identified 8 transitions in a phylogenetically diverse sample of 63 species [Hillis and Green, 1990]. However, the same data also show that many species of fish and amphibians have not undergone a change in sex determination since more than several million years ago, and yet, almost none of these species show evidence of sex chromosome differentiation [Stöck et al., 2011].

A plausible explanation for the maintenance of homomorphic sex chromosomes in lower vertebrates was pro-

posed by Perrin [2009]. According to his fountain-of-youth hypothesis, the sex chromosomes of cold-blooded vertebrates are prevented from degenerating due to rare recombination events prompted by a low rate of sex reversal (ectotherms are liable to rare sex reversal under natural conditions, due to the general impact of temperature on their development). Consistent with this idea, a recent analysis of neutral genetic variation on the sex chromosomes provides statistical support for a low rate of X-Y recombination in European tree frogs (*Hyla* spp.) [Guerrero et al., 2012b], a group of species with cryptic sex chromosomes and stable sex determination [Stöck et al., 2011]. Furthermore, a theoretical study of mutation accumulation on the sex chromosomes demonstrates that occasional sex reversal events provide sufficient opportunity for recombination to purge the Y chromosome from its deleterious mutation load [Grossen et al., 2012]. The evolution of sex chromosomes (or the lack thereof in species with ESD) has several other consequences for the genetic architecture that can interact with the evolution of sex determination. Some of these are discussed by Ewert and Nelson [1991] (inbreeding), Reeve and Pfennig [2003] (sexual selection) and Kitano and Peichel [2012] (speciation).

A central challenge for future work is to combine our understanding of the population genetics of sex determination transitions with molecular data [Pomiankowksi et al., 2004]. Most of the models discussed in this review make specific assumptions about the developmental mechanisms implementing sex determination, but these are not always explicitly stated as such. Models that incorporate the available knowledge of sex determination pathways are essential to complement the black box approach of population-genetic analysis, to validate its implicit assumptions and to generate testable predictions. For example, combining evolutionary and systems biology modeling approaches can help to clarify why it is so rare to observe 'leaky' sex determination. Is it because selection generally disfavors (partially) random sex determination or is a canalized all-or-nothing switch easier to implement, considering that the developmental decision triggered by the initial sex determination signal must be stabilized and retained during development [Valenzuela, 2008]? This issue is relevant for some sex ratio selection models of sex determination transitions, as these assume that developmental constraints prevent optimization of the sex ratio in species with single-locus GSD. A second possibility is to look into how sex determination interacts with downstream developmental processes and how these interactions generate potential pleiotropic fitness effects. The results of such an analysis may validate the common assumption that sex determination can be studied in isolation from other developmental processes (which means that sex determination modifiers can safely be assumed to have no other fitness effects). Alternatively, they could support the pleiotropic benefit hypothesis for the evolution of sex-determining genes.

Finally, realistic models of evolving sex-determining pathways are needed to clarify how selection and molecular mechanisms interact to generate the variation of evolutionary rates between genes at different positions in the sex-determining cascade [MacCarthy et al., 2010]. Despite that population-genetic models tend to ignore the complexity of development, they do offer suggestions for why the flexibility of sex determination may predominantly rely on the recruitment of novel upstream regulators to the pathway. In particular, it seems reasonable to suppose that a novel upstream regulator that interacts directly with the original master switch (for example, by mimicking or blocking the primary sex determination signal) can reverse the outcome of the original switch for a certain set of environmental or genetic conditions and, in this way, act as an epistatically dominant modifier of sex determination. By contrast, regulators interacting with downstream sex determination genes would perhaps interfere with how the sex determination signal is processed, which would be likely to have negative pleiotropic effects or result in a breakdown of canalization [Uller and Helanterä, 2011]. Selection against leaky sex determination may therefore be one of the factors preventing the spread of downstream sex determination modifiers. A thorough test of this hypothesis requires investigating how genes in the sex determination pathway coevolve. There is growing evidence that such coevolution is occurring [Haag and Ackerman, 2005; Stothard and Pilgrim, 2006] and that it is associated with rapid evolution of sex-determining genes, often without sex determination transitions or structural pathway rearrangements [Ferris et al., 1997; O'Neill and O'Neill, 1999; Chandler et al., 2012]. The evolutionary flexibility of sex determination may therefore be even higher than is currently appreciated.

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