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Genes, environment and sex: perspectives from ectothermic vertebrates

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SUMMARY

Classical models of sex-chromosome evolution assume that sexually antagonistic genes accumulate on sex chromosomes leading to a non-recombining region, which progressively expands and favors the accumulation of deleterious mutations. Concordant with this theory, sex chromosomes in extant mammals and birds are considerably differentiated. In most ectothermic vertebrates, such as frogs, however, sex chromosomes are undifferentiated and a striking diversity of sex determination systems is observed. This thesis was aimed to investigate this apparent contrast of sex chromosome evolution between endothermic and ectothermic vertebrates. The "high-turnover" hypothesis holds that sex chromosomes arose regularly from autosomes preventing decay. The "fountain-of-youth" hypothesis posits that sex chromosomes undergo episodic X-Y recombination in sex-reversed XY females, thereby purging ("rejuvenating") the Y chromosome. We suggest that both processes likely played an important role in sex chromosome evolution of ectothermic vertebrates. The literature largely views sex determination as a dichotomous process: individual sex is assumed to be determined either by genetic (genotypic sex determination, GSD) or by environmental factors (environmental sex determination, ESD), most often temperature (temperature sex determination, TSD). We endorsed an alternative view, which sees GSD and TSD as the ends of a continuum. The conservatism of molecular processes among different systems of sex determination strongly supports the continuum view. We proposed to define sex as a threshold trait underlain by a liability factor, and reaction norms allowing modeling interactions between genotypic and temperature effects. We showed that temperature changes (due to e.g., climatic changes or range expansions) are expected to provoke turnovers in sex-determination mechanisms maintaining homomorphic sex chromosomes. The balanced lethal system of crested newts might be the result of such a sex determination turnover, originating from two variants of ancient Y-chromosomes. Observations from a group of tree frogs, on the other hand, supported the 'fountain of youth' hypothesis. We then showed that low rates of sex-reversals in species with GSD might actually be adaptive considering joint effects of deleterious mutation purging and sexually antagonistic selection. Ongoing climatic changes are expected to threaten species with TSD by biasing population sex ratios. In contrast, species with GSD are implicitly assumed immune against such changes, because genetic systems are thought to necessarily produce even sex ratios. We showed that this assumption may be wrong and that sex-ratio biases by climatic changes may represent a previously unrecognized extinction threat for some GSD species.

RÉSUMÉ

Les modèles classiques sur l'évolution des chromosomes sexuels supposent que des gènes sexe-antagonistes s'accumulent sur les chromosomes sexuels, entraînant ainsi l'apparition d'une région non-recombinante, qui se répand progressivement en favorisant l'accumulation de mutations délétères. En accord avec cette théorie, les chromosomes sexuels que l'on observe aujourd'hui chez les mammifères et les oiseaux sont considérablement différenciés. En revanche, chez la plupart des vertébrés ectothermes, les chromosomes sexuels sont indifférenciés et il existe une impressionnante diversité de mécanismes de détermination du sexe. Au cours de cette thèse, j'ai étudié l'évolution des chromosomes sexuels chez les vertébrés ectothermes, en outre pour mieux comprendre ce contraste avec les vertébrés endothermes. L'hypothèse « high-turnover » postule que les chromosomes sexuels sont remplacés régulièrement à partir d'autosomes afin d'éviter leur dégénérescence. L'hypothèse « fountain-of-youth » propose que la recombinaison entre le chromosome X et le chromosome Y au sein de femelles XY empêche la dégénérescence. Les résultats de ma thèse, basés sur des études théoriques et empiriques, suggèrent que les deux processus peuvent être entraînés par l'environnement et ainsi jouent un rôle important dans l'évolution des chromosomes sexuels chez les vertébrés ectothermes.

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INTRODUCTION

Sex determination, the decision whether an individual will be male or female, is a fundamental process during the development of an individual. In most species, sex determination is irreversible as it generally causes complex morphological changes. Fundamental developmental processes are expected to be subject of strong selection, reducing genetic variation. At the population and species level, sex determination plays an essential role, because in many animal and plant species, both sexes are indispensable for reproduction. Therefore, sex determination is expected to be a conserved process among different organisms. The developmental path of sex differentiation, such as the structural organization of testes and ovaries, is indeed well conserved across vertebrates, where gonads arising from bilateral ridges are capable to develop either into ovaries or testes. The developmental switch triggering sex determination, however, is astonishingly diverse across species (Bull 1983). To produce different sexes among organisms, either part of the genes must differ among the individuals or the individuals experience different environments. Genetic sex determination is either based on several genes of major (multifactorial) or minor (polygenic) effect or one major gene (monogenic, Bull 1983). The latter may lead to the evolution of differentiated sex chromosomes as it is observed in mammals and birds. Most mammals have a monogenic *XX*/*XY* sex determination system, except in a few species where the Y chromosome has been lost. Among birds, sex is determined by one major gene, with females being the heterogametic sex (*ZW*) and the male being the homogametic sex (*ZZ*). Furthermore, the environment plays a crucial role in sex determination of many vertebrates. Under environmental sex determination (ESD) the sex is determined by the environment during a specific period of embryonic development. A considerable number of environmental factors have been shown to influence sex determination in a variety of species. Temperature is the most frequently observed environmental factor, but also pH, day length, nutrient availability and even social context dependent sex determination have been observed (Bull 1983; Francis & Barlow 1993; Romer & Beisenherz 1996).

ESD AND GSD: TWO EXTREMES OF A CONTINUUM

Sex determination is often seen as a dichotomy; either genotypic or environmental, as two opposing ways that sex may be determined (Valenzuela, Adams, & Janzen 2003; Ospina-Álvarez & Piferrer 2008). But genetic sex determination (GSD) and environmental sex determination (ESD) may rather be seen as two ends of a continuum (Sarre, Georges, & Quinn 2004). On one end is pure GSD, sex being determined fully by genetic components, and on the other end is ESD, with sex being solely determined by the environment. Under ESD, only one major genotype for genes influencing sex determination is present in the population. The sex of an individual can not be predicted from its genotype. In mixed sex determination systems, genotype and the environment

interact to determine the sex of an individual, with either the environment or genotype playing a slightly more important role. Mixed systems were observed in several reptile and fish species (e.g. Conover & Heins 1987; Shine, Elphick, & Donnellan 2002; Quinn et al. 2007; Baroiller et al. 2009a; Pen et al. 2010).

The view that sex determination should rather be seen as a continuum between ESD and GSD is not only based on the many observations of mixed sex determination, but also on the growing evidence that molecular pathways are conserved among very diverse sex determination systems (Raymond et al. 1998; Schartl 2004; Graves & Peichel 2010). Homologous sex determining genes and sex chromosomes were observed in diverse taxa with seemingly very different sex determination systems. The same sets of genes were shown to determine whether a bipotential gonad will develop either into male or female organs. For instance, the transcription factor *DMRT1* plays an important role in vertebrate sex determination (Ferguson-Smith 2007). The DM-domain, originally described in *C. elegans* and *Drosophila melanogaster*, plays a crucial role in the male determination cascade throughout all vertebrates (Hodgkin 2002; Haag & Doty 2005), including both GSD (Raymond et al. 2000; Ounap et al. 2004; Yoshimoto et al. 2008; Smith et al. 2009) and TSD (temperature-dependent sex determination) species (Kettlewell, Raymond, & Zarkower 2000; Shoemaker et al. 2007). In humans, haploinsufficiency (only one copy of the gene functional) of the *DMRT1* region has been related to *XY* female sex reversals (Raymond et al. 1999). In the medaka fish (*Oryzias latipes*), new sex chromosomes evolved recently through the duplication of the autosomal copy of *DMRT1* (Matsuda et al. 2002; Nanda et al. 2002). High temperatures (32°C) were been shown to up-regulate the autosomal *DMRT1* copy, whereby a significant fraction of *XX* individuals developed into fertile males (Sato et al. 2005; Hattori et al. 2007). Hence, the expression level of the transcription factor *DMRT1* (depending on a combination of genetic and environmental effects) determines the sex in medaka. *DMRT1* was also shown to be the strongest candidate for the sex-determining gene in birds. It is located on the Z and absent from the W and the expression is double in *ZZ* males compared to the *ZW* females suggesting a dosage-related mechanism (Smith et al. 2009). The autosomal gene *SOX9* may also play a crucial role in sex determination of all vertebrates (including both GSD and TSD species, Bagheri-Fam, Sinclair, & Koopman 2010). The gene is found in both sexes, but it is up regulated in the male pathway. Studies with *SOX9* deficient mice showed that expression of *SOX9* was necessary for testis formation (Kobayashi 2005).

The developmental switch triggering a mammal, bird, reptile, amphibian or fish to develop into a male or female largely relies on the hormonal environment of the developing embryo. Hormones may promote either testes or ovaries (androgenic or estrogenic). Therefore it is unsurprising that hormone-induced sex reversals are effective across vertebrates (reviewed in Nakamura 2010). As the amount of sex factor determining the sex of an

individual may be influenced by genes, the environment or the joint action of both, what are the consequences for the evolution of sex chromosomes?

EVOLUTION OF SEX CHROMOSOMES

The first step of sex chromosome evolution from autosomes is generally seen to be set by the evolution of a gene with two alleles g and G, where G is a dominant gender-determinant, heterozygotes *gG* developing into one sex, homozygotes *gg* into the other (Rice 1996). Recombination suppression around the sex determiner(s) leads to the evolution of X and Y-chromosomes. Alleles conferring male advantage (e.g. bright colors in guppies, Lindholm & Breden 2002) will accumulate close to the new sex determiner (Bull 1983; Rice 1987; 1996), further increasing selection against recombination between the allele conferring male advantage and the sex determining genes. This accumulation of sexually antagonistic genes (Rice 1992) and the possibility of evolving localized recombination suppression (most likely by recombination modifier genes, Chinnici 1971) was shown experimentally in *Drosophila*. An alternative solution to allow sexual dimorphism without producing low-quality males or females due to recombination, would be sex-limited expression of sexually antagonistic genes, but this is likely more difficult to evolve (Rice 1996 and references therein). Further genes with either a sex-specific function or with sexually antagonistic effects will accumulate and advance recombination suppression. By this process, the non-recombining segment on the chromosome increases stepwise. Strata on extant sex chromosomes are evidence for the stepwise breakdown in recombination around the sex determination genes. Several recombination cessation events have been observed in plants, mammals and birds (Lahn & Page 1999; Lawson-Handley, Ceplitis, & Ellegren 2004; Nicolas et al. 2005; Fraser & Heitman 2005).

Reduced recombination between the two sex chromosomes favors the accumulation of deletions, insertions, duplications and rearrangements, further suppressing recombination. The non-recombining sex chromosome (i.e. the Y or W chromosome) is expected to undergo mutational decay. Dosage compensation may further accelerate deleterious mutation accumulation on the non-recombining chromosome by lowering selection pressures on its genes (Charlesworth 1978; Engelstaedter 2008). Furthermore, the effective population size of the non-recombining region of Y (or W) is four times smaller than for the autosomes and selection affecting one locus affects others linked to it, further reducing the effective population size, hence Y-chromosomes are more affected by drift. Both the Y and the W have empirically been shown to have highly reduced genetic diversity compared to autosomes in a variety of organisms (Bachtrog 2000; Filatov et al. 2000;

Berlin & Ellegren 2004; Rozen, Marszalek, & Alagappan 2009). The joint action of genetic drift, selective sweeps, background selection and Muller's ratchet leads to the degeneration (Rice 1996; Charlesworth & Charlesworth 2000; Charlesworth, Charlesworth, & Marais 2005; Graves 2006; Engelstaedter 2008; Ellegren 2011) or even loss of the non-recombining chromosome (e.g. *Ellobius lutescens*, Just et al. 1995),

As a consequence of this combination of inefficient selection and accidental loss of genes, mammalian sex chromosomes are highly dimorphic and recombination is restricted to a small homologous region (the pseudoautosomal region). The human Y, for instance, is much smaller than the X, with less than 100 functional genes (presumably conferring vital sex-specific functions) compared to about 1000 functional genes on the X (Graves 2006 and references therein). As the loss of genes happened independently after the formation of the proto-Y, different mammal lineages have lost different subsets of genes (Figure 5 in Graves, 2006). Even among humans, there are different variants of Y-chromosomes: a family of Y-chromosomes widespread in Europe has a deletion of 1.8 Mb covering eight testis-specific gene families. Deletion of these gene families had either low fitness effects or were counterbalanced by another factor (Repping et al. 2004). The W chromosome of birds has also degenerated, most of all in neognathous birds (such as the chicken), but less so in paleognathous birds (for instance emu, Ezaz, Stiglec, et al. 2006a). Only a small subset of all the genes found on the chicken Z are also found on the W (Fridolfsson et al. 1998). Similar observations come from studies of *Drosophila* species. The Y of *Drosophila miranda* evolved about one million years ago and a substantial proportion of genes degenerated (Steinemann & Steinemann 1998; Bachtrog 2003b). Chromosomal rearrangements included transposition of autosomal genes into the Y, as has been observed in mammals (Skaletsky et al. 2003). In *Drosophila melanogaster*, only genes with male function have been found on the Y. None of these genes were found to have a copy on the X, hence they presumably originated from autosomes (reviewed in Carvalho, Koerich, & Clark 2009). Transposable elements are generally predicted to accumulate in non-recombining regions and have also been shown to play an important role in sex chromosome evolution. Transposition events of transposable elements can have deleterious effects; for instance when inserting themselves into a gene interrupting its function. Accumulation of transposable elements has for instance been shown in medaka (*Oryzias latipes*, Nanda et al. 2002) and three-spined sticklebacks (*Gasterosteus aculeatus*, Peichel et al. 2004). Nearly 50% of the DNA content on the Y of *Drosophila miranda* are transposable elements (Bachtrog 2003a). The evolution of highly specialized sex determination systems with rearranged, mostly non-recombining Y (or W) chromosomes is very unlikely to be reversible and consequently sex determination systems are expected to be evolutionarily stable. Long-term stability in sex determination systems was indeed shown to be the case in mammals and birds. Sex chromosomes

of mammals, *Drosophila* and birds are probably the best studied and theory about sex chromosome evolution is strongly founded on these observations, most of all the male heterogametic systems of mammals and *Drosophila*.

Sex determination in the remaining groups of vertebrates severely complicates the picture, raising the question whether the classical models of sex chromosome evolution described above are applicable for all animals. An astonishing diversity of sex determination systems is observed in ectothermic vertebrates, fish, reptiles and amphibians (see e.g. Schartl 2004). Sex may be determined by male or female heterogamety, by one or several different genes, the environment (most notably temperature) or the interaction between different systems (mixed systems). Closely related species may have very different sex determination systems. Even within populations of the same species as for instance observed in the wrinkled frog *Rana rugosa* (Miura 2008) sex determination may differ. Frequent transitions and evolutionary lability of sex determining systems have been shown for reptiles (Ezaz, Stiglec, et al. 2006a) as well as for fish (reviewed in Devlin & Nagahama 2002; Schartl 2004; Mank & Avise 2009). The latter shows the highest variety of SD mechanisms among vertebrates, including hermaphrodites and multiple sex chromosomes. Sex chromosome differentiation in amphibians, reptiles and fish is generally very low and sex chromosomes are mostly homomorphic (Devlin & Nagahama 2002; Eggert 2004 and references therein). In amphibians for instance, only 4% out of 500 species studied showed heteromorphic sex chromosomes (Eggert 2004) and in fish, about 10% of the examined species, were found to have differentiated sex chromosomes (Devlin & Nagahama 2002). Differentiated sex chromosomes are slightly more frequent among reptiles; with for instance less than 20% differentiated sex chromosomes in species karyotyped in lizards (Olmo 1986; Ezaz, Sarre, et al. 2009a). In striking contrast, birds and mammals have often highly differentiated sex chromosomes (Ezaz, Stiglec, et al. 2006a; Graves 2006).

What does that imply for the generality of the model of sex chromosome evolution? Is it also valid for ectothermic vertebrates? The general model of sex chromosome evolution predicts that X-Y divergence correlates with the age of sex chromosomes. Therefore, homomorphic sex chromosomes are expected to be of a recent origin. Based on this prediction, sex chromosomes of most ectothermic vertebrates should have evolved much more recently than sex chromosomes of most mammals and birds. Is this prediction valid or are there alternative explanations? At least two mechanisms have been proposed to explain how sex chromosomes may be maintained homomorphic and how this striking difference between sex determination systems in vertebrates may have evolved: Frequent turnovers of sex determination systems and the fountain-of-youth hypothesis.

TURNOVER HYPOTHESIS

A high turnover of sex chromosomes is generally given as explanation for a lack of sex chromosome differentiation. A sex chromosome turnover happens, when a new master sex-determiner appears on an autosome and replaces the previous sex chromosomes. If new sex chromosomes arise regularly from autosomes, extant sex chromosomes don't have time to accumulate deleterious mutations or structural changes. Consequently, regular sex determination turnovers maintain 'young' and homomorphic sex chromosomes. The high diversity of sex determination systems together with the prevalence of homomorphic sex chromosomes in ectothermic vertebrates supports the turnover hypothesis well (Volff et al. 2007). Sex determination turnovers seem to be especially frequent in the fly *Megaselia scalaris*, in which the sex-determining gene might be carried by a transposable element and is located on different chromosomes depending on the population (Traut & Willhoeft 1990). Recent turnovers in vertebrates are found for instance in the three-spined stickleback (Peichel et al. 2004; Ross et al. 2009), the medaka *Oryzia latipes* (involving a copy of *DMRT1*, Matsuda et al. 2002; Nanda et al. 2002; Kondo et al. 2004; Tanaka et al. 2007) and *Rana rugosa* (Miura 2008). If turnovers are indeed frequent in ectothermic vertebrates and may explain the maintenance of homomorphic sex chromosomes, what causes these turnovers? Several mechanisms have been proposed to explain turnovers: New sex determiners may spread and invade (i) if the new sex genotypes have a higher adaptive value than the previous sex genotype (Bull & Charnov 1977; Orzack et al. 1980; Basolo 2001; Kraak & Pen 2002), (ii) if a genetic conflict arising from sex-chromosome meiotic drive or cytoplasmic sex-ratio distorters favors a turnover (e.g. *Wolbachia*, Hamilton 1967; Werren & Beukeboom 1998; Caubet et al. 2000), (iii) if changes in the production costs of male and female offspring lead to sex-ratio selection (Kozielska et al. 2006), or (iv) if strong sexually antagonistic genes are linked to a new sex determiner (van Doorn & Kirkpatrick 2007). However, none of these models account for the striking differences between ectothermic and endothermic vertebrates.

Temperature-dependent sex determination (TSD), mixed systems and temperature-induced sex reversals were shown to be widespread among ectothermic vertebrates. The influence of temperature on a large number of physiological processes including sex determination could be linked to the high diversity in sex determination systems and the high frequency of homomorphic sex chromosomes in ectothermic vertebrates. In **chapter one** of this thesis, we developed a model for sex determination simultaneously accounting for both, genetic and environmental effects. We then used this model to investigate if climatic changes might trigger the turnover of sex determination systems, *Temperature-dependent turnovers in sex-determination mechanisms: a quantitative model*.

The model developed in chapter one was then applied to a case study in **Chapter two**: *The balanced lethal system of crested newts.* A sex determination turnover (for instance induced by climatic changes) is proposed to have led to the evolution of the balanced lethal system in crested newts *Triturus cristatus*.

A drawback of the turnover hypothesis is, that the high proportion of homomorphic sex chromosomes in ectotherms would imply very frequent turnovers. However, the diversity of sex determination systems observed in amphibians may be explained by approximately seven transitions (Hillis & Green 1990). Such a low number of transitions is unlikely to account for the fact that 96% of sex chromosomes in amphibians are homomorphic.

FOUNTAIN-OF-YOUTH HYPOTHESIS

The "fountain-of-youth" hypothesis (Perrin 2009) proposes an alternative to the frequent turnover hypothesis. It states that even, if X and Y (or Z and W) normally do not recombine among each other, there might be sporadic recombination events allowing deleterious mutations to be purged. The underlying assumption is that suppression of recombination is specific to phenotypic sex, not heterogamety. If sex reversals are possible in a species, a genotypic male becoming a *XY* female may recombine similarly to normal *XX* females. However, sex-reversed *XX* males are expected to not recombine (at least not between sex determining and sexually antagonistic genes). Newts, medaka and the common frog (Wallace, Wallace, & Badawy 1997; Kondo et al. 2001; Matsuba, Alho, & Merila 2010) show evidence for this phenomenon. Sporadic sex-reversals (for instance induced by temperature) lead to *XY* females (or *ZW* males) and, thereby, provide the opportunity for recombination between the two different, normally non-recombining chromosomes. Sex-reversal may, therefore, improve the purging on the Y (or W). As a consequence, the Y is less likely to degenerate. Sex chromosomes are expected to remain homomorphic even if the sex chromosomes are of ancient origin and lack recombination in the heterogametic sex. Selection may favor sex reversal in the heterogametic sex in order to purge deleterious mutations. Chapter three, *Ever-young sex chromosomes in European tree frogs*, a study on sex chromosome evolution in three tree frog species, provides an empirical test of this hypothesis.

Sexually antagonistic selection is expected to counteract sex-reversal and recombination for two reasons: (i) sex reversed individuals have a lowered fitness, because their genotype at the sexually antagonistic genes does not match their phenotypic sex and (ii) sex reversals leading to recombination disrupt epistatic interactions between the sex determiner and sex-antagonistic alleles. Purging by sex-reversal is therefore, expected to be counteracted by sexually antagonistic selection. The importance of these opposing selective

pressures for the evolution of the rate of sex reversal including advantages and disadvantages of GSD and TSD, were investigated in **Chapter four**, *Evolutionarily stable rates of sex reversal*.

In **Chapter five**, we explore the advantages and disadvantages of GSD and TSD in a more applied context. Species with TSD were often proposed as being at risk due to climatic changes (e.g. Witt et al. 2010; Fuentes, Hamann, & Limpus 2010; Mitchell & Janzen 2010). In Chapter five, *Extinction risks under climatic changes: what role for sex determination mechanisms?,* we reviewed the general temperature dependence of sex determination in ectothermic vertebrates. Furthermore, we discussed the consequences for species with genetic sex determination, which might also be at risk.

CHAPTER I

TEMPERATURE-DEPENDENT TURNOVERS IN SEX-DETERMINATION MECHANISMS: A QUANTITATIVE MODEL

Christine Grossen, Samuel Neuenschwander and Nicolas Perrin

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ABSTRACT

Sex determination is often seen as a dichotomous process: individual sex is assumed to be determined either by genetic (genotypic sex de termination, GSD) or by environmental factors (environmental sex determination, ESD), most often temperature (temperature sex determination, TSD). We endorse an alternative view, which sees GSD and TSD as the ends of a continuum. Both effects interact a priori, because temperature can affect gene expression at any step along the sex-determination cascade. We propose to define sex-determination systems at the population- (rather than individual) level, via the proportion of variance in phenotypic sex stemming from genetic versus environmental factors, and we formalize this concept in a quantitative-genetics framework. Sex is seen as a threshold trait underlain by a liability factor, and reaction norms allow modeling interactions between genotypic and temperature effects (seen as the necessary consequences of thermodynamic constraints on the underlying physiological processes). As this formalization shows, temperature changes (due to e.g., climatic changes or range expansions) are expected to provoke turnovers in sex- determination mechanisms, by inducing large-scale sex reversal and thereby sex-ratio selection for alternative sex-determining genes. The frequency of turnovers and prevalence of homomorphic sex chromosomes in cold-blooded vertebrates might thus directly relate to the temperature dependence in sex-determination mechanisms.

INTRODUCTION

Birds and mammals display a strictly genotypic sex determination (GSD), with highly differentiated sex chromosomes (Graves 2008). The *XX/XY* male-heterogametic system of mammals has remained stable since the master male-determining gene SRY first appeared close to 200 million years ago (Potrzebowski et al. 2008; Veyrunes et al. 2008). Loss of recombination has since induced strong differentiation and degeneration of the Y chromosome. A similar process occurred in birds, where females are the heterogametic sex and thus carry the decayed W chromosome (*ZW/ZZ* system).

Patterns are strikingly different in other vertebrates. First, sex determination is often extremely labile. Different sex-determination systems may be found in closely related taxa (Hillis & Green 1990; Ezaz, Stiglec, et al. 2006a; Baroiller et al. 2009a) sometimes even in different populations from the same species (Miura et al. 1998). Transitions between systems seem frequent on an evolutionary time scale (Hillis & Green 1990; Janzen & Krenz 2004; Ezaz, Stiglec, et al. 2006a; Mank, Promislow, & Avise 2006; Graves 2008) with the result that sex chromosomes (here defined as chromosomes bearing sex-determining genes, independent of recombination patterns), are usually homomorphic or poorly differentiated (e.g., Hillis & Green 1990; Janzen & Krenz 2004; Ezaz, Stiglec, et al. 2006a; Mank et al. 2006; Graves 2008).

Second, the environment also plays a role, temperature in particular. Sex may be determined mostly by temperature (such as found in turtles or alligators) or by the joint action of temperature and genetic factors (Bull 1980; Conover & Heins 1987; Conover, Voorhees, & Ehtisham 1992; Janzen & Phillips 2006). There is mounting evidence for mixed sex determination systems (where GSD may be overridden by temperature within the natural range) in lizards (Shine et al. 2002; Quinn et al. 2007; Radder et al. 2008) and fish (reviewed in Ospina-Álvarez & Piferrer 2008; Baroiller et al. 2009a), including species with differentiated sex chromosomes. Sex reversal also occurs spontaneously in species considered to have purely GSD (e.g. Crew 1921; Witschi 1929a; b; Aida 1936; Kawamura & Nishioka 1977; Nagler et al. 2001; Matsuba, Miura, & Merila 2008). Even when sex determination is strictly genotypic under natural conditions, temperature has been shown to play a role in experimental conditions (Witschi 1929b; Wallace & Wallace 2000; Eggert 2004; Ospina-Álvarez & Piferrer 2008). Temperature effects are probably not adaptive in such cases, but more likely the side effect of a temperature-dependence in underlying molecular processes.

Two highly contrasted views exist regarding interactions between, respectively, temperature sex determination (TSD) and GSD. The conventional view sees sex determination as a dichotomous process. As

formulated by Valenzuela (2003), the sex of organisms is determined by two distinct and mutually exclusive mechanisms. In GSD, sex is determined at conception by genes, whereas in environmental sex determination (ESD, as for instance TSD), sex is determined after fertilization by environmental factors. To account for intermediate situations (where both genes and environment interact), Valenzuela et al. (2003) propose that (1) some species can be categorized as GSD + EE: that is, sex is determined at conception by genes, but may be then reversed by environment during the embryonic development; (2) TSD species may harbor genetic variance in their sensitivity to environment, but are still to be considered as TSD, because at the individual-level sex is determined by environment; (3) even when TSD and GSD coexist within species or populations, at the individual level, sex is still determined either by genes or by the environment (Valenzuela et al. 2003).

The alternative view, by contrast, sees GSD and TSD as the two ends of a continuum (e.g., Sarre et al. 2004). The observed continuity in phenotypic patterns of sex determination reflects the extraordinary conservatism in the gonadal developmental pathways of vertebrates, as revealed by recent molecular studies. In both TSD and GSD species, the same genes are involved in the cascade of processes that result in sex determination (Sarre et al. 2004). At each stage along this path, temperature may affect molecular processes, and thereby the final outcome. Temperature is known, for instance, to affect the activity of enzymes (such as aromatase, which transforms testosterone into estradiol; e.g., (Desvages, Girondot, & Pieau 1993; Crews & Bergeron 1994; Crews et al. 2001; D'Cotta et al. 2001; Lance 2009) or the expression of genes (such as DMRT1) involved in the sex-determining cascade.

This may be illustrated by the case of medaka fish (*Oryzias latipes*). New sex chromosomes recently evolved in this lineage through the duplication of DMRT1 (Matsuda et al. 2002; Nanda et al. 2002), a gene encoding a transcription factor with a DM-domain playing a crucial role in the male determination cascade throughout all vertebrates (Hodgkin 2002; Haag & Doty 2005), including both GSD (Raymond et al. 2000; Ounap et al. 2004; Yoshimoto et al. 2008; Smith et al. 2009) and TSD species (e.g., Kettlewell et al. 2000; Shoemaker et al. 2007). At normal temperatures (25°C), DMRT1 expression from the autosomal copy is too low to reach the threshold required to induce male development, so that *XX* individuals develop into females. By contrast, *XY* individuals develop into males due to the additional expression of the duplicated copy on the proto Y (DMRT1bY). Higher temperatures (32°C), however, upregulate the autosomal DMRT1 copy, so that a significant fraction of *XX* individuals develop into males, which are perfectly functional and phenotypically indistinguishable from *XY* males (e.g., Sato et al. 2005; Hattori et al. 2007). Sex is thus determined by the amount of DMRT1 transcription factor, which itself depends on a combination of genetic and environmental effects. Interestingly,

DMRT1bY is first expressed at the neurula stage (stages 17–18; Nanda et al. 2002), which coincides temporally with the thermally sensitive period of masculinization (Hattori et al. 2007). Hence, opposing the conventional definition (Valenzuela et al. 2003), the timing of sex determination does not necessarily differ between TSD and GSD.

In a quantitative-genetics perspective, sex can be seen as a threshold trait that depends on an underlying liability factor (e.g., DMRT1 expression in medaka), which is under both genetic and environmental influence (e.g., Bull 1981; Bulmer & Bull 1982; Roff 1996). Under this concept, an individual trait value cannot be assigned to either genes or environment. Why does an *XX* medaka fish become male at 32°C? Sex determination is neither purely genetic (as temperature plays an obvious role), nor purely environmental (as with different autosomal DMRT1 alleles, the individual might have developed into a female) but results from an interaction between the two factors (temperature dependence of DMRT1 expression). The relevant question in quantitative genetics is that of the apportionment of phenotypic variance within populations, and this directly relates to the definition of sex-determination systems. In line with the alternative view, we propose to define a system as GSD if all (or most) of the variance in sex determination within the normal environmental range can be assigned to genetic factors, and ESD if all (or most) of this variance can be assigned to environmental factors. These are to be seen as special cases of a more general situation, where phenotypic variance has both genetic and environmental components. This definition obviously differs from the one proposed by the conventional view.

An appealing way to formalize gene–environment interactions in this unifying view is to represent genotypes as reaction norms in the space defined by phenotype versus environment (Fig. 1). In any given environment, different genotypes may express large differences (major genes) or small differences (minor genes) in liability-trait values. Environmental effects define the shape of the norm (e.g., thermal up-regulation of DMRT1 expression) which may be linear or not, and may or not vary among genotypes (G × E interactions). Environmental effects are to be seen as constraints stemming from the laws of thermodynamics. Thermal upregulation of aromatase inhibitors in tilapia (D'Cotta et al. 2001), or DMRT1 expression in medaka (Hattori et al. 2007), for instance, are seen as the necessary consequences of increased kinetic energy in the underlying physico-chemical processes. In GSD species, this effect of temperature on the liability trait might be hidden by genes with major effects (i.e., the liability-trait values expressed by different genotypes are so far apart from the threshold that sex is under complete genetic control within the natural range). TSD species, by contrast, have capitalized on this constraint to make temperature dependence a functional system. This evolution clearly required a set of specific co-adaptations (regarding e.g., nest-choice behavior or the shape of reaction norms) to

render this strategy adaptive. The evolution of TSD is certainly a fascinating question, but one outside the scope of the present article.

The first aim of our formalization effort is to show that the alternative paradigm, in which sex determination depends on both genes and environment, directly accounts for the frequent transitions occurring between sex-determination systems. If a population of medaka fish were to live in a consistently warm habitat, where a majority of *XX* individuals develop into males, then the ensuing sex-ratio bias will necessarily induce a strong selective pressure for a new sex-determining system. This might be achieved, for example, by the appearance of a new allele on the autosomal DMRT1 copy that downregulates its expression. Individuals with this copy would develop into females, so the system would have evolved toward female heterogamety through a sex-chromosome turnover.

The second point we want to make is that this process may help explaining why transitions seem more frequent in cold-blooded than in warm-blooded vertebrates. In the formers, the developing embryos are under the direct influence of external temperature, which will necessarily interfere with sex determination owing to underlying thermodynamic constraints. Hence, temperature drifts (induced e.g., by climatic changes or range expansions) will necessarily cause turnovers. In the warm-blooded birds and mammals, by contrast, embryonic temperature is kept constant by parental control during the sensitive period of development, which will prevent sex-reversal and the ensuing sex-ratio selection for turnovers. These groups are thus expected to display evolutionarily stable GSD.

CONCEPTUAL MODEL

Phenotypic distribution of the liability trait

Let us consider one master sex-determining locus and two alleles X and Y with additive effects, X having a feminizing effect and Y a masculinizing effect (i.e., Y produces more of the liability trait *A* than does X). Depending on the strengths of these effects relative to the threshold value, the model may account for either male heterogamety (*XX* females, *XY* males) or female heterogamety (*XY* females, *YY* males). In both cases the two genotypes involved constitute a recurrent pair (sensu Bull 1983), that is, two genotypes of opposite sex, which when mated produce only the same two parental genotypes, in a 1:1 ratio.

Masculinization or feminization effects also depend on temperature. Each genotype *IJ* (I, J = X, Y) is characterized by a norm of reaction, defining its liability-trait value, $A_{IJ,T}$ (Table 1), as a function of temperature *T*. Such a norm can be represented as a curve in the space phenotype-environment, assumed here to be linear with slope β (Fig. 1). The genetic component of the phenotypic variance in *A* within populations comes from the coexistence of different genotypes, producing different *A* values at any given temperature. The environmental component stems from the fact that different individuals with the same genotype experience slightly different microenvironments, and thus have different values of the liability trait (Fig. 1).

Assuming normal distributions for environmental deviations from genotypic averages (with standard deviation σ_E), the phenotypic distribution of the liability trait *A* within populations, at any average temperature *T*, is the sum of several normal distributions (one per genotype) weighted by genotypic frequencies.

Sex determination, sex reversal and sex ratios

Sex is considered as a threshold trait, that is, there is a threshold value (ζ) for the liability trait *A*, such that for each genotype *IJ*, a proportion $r_{IJ,T}$ exceeds the threshold and develops into males, whereas the complementary proportion $1 - r_{IJ,T}$ develops into females. Pure GSD will result if, at given *T*, liability-trait values ($A_{IJ,T}$) are far apart from the threshold, and σ_E is low. In such a case, phenotypic sex perfectly correlates with genotypes. In contrast, pure TSD will result if a single genotype is fixed, and phenotypic variance in *A* only results from environmental variation. In between these two extreme cases, the correlation between phenotypic sex and genotypes will be imperfect, with a variable amount of sex-reversed individuals. The relevant quantity to determine the amount of sex reversal for genotype *IJ* at temperature *T* is the distance of its liability value to the threshold ζ, in units of σ_E. Hence it is useful to standardize these values as $\alpha_{IJ,T} = \frac{A_{IJ,T} - \zeta}{\sigma_E}$. Using this metrics, the slope of the reaction norm is expressed in units of standard errors (β/σ_E) and the proportion of males among individuals of genotype *IJ* becomes $r_{IJ,T} = \frac{1}{2}\left(1 + erf\left(\frac{\alpha_{IJ,T}}{\sqrt{2}}\right)\right)$, where $erf(x) = \frac{2}{\sqrt{\pi}}\int_{z=0}^{x} e^{-z^2}\,dz$ is the so-called error function. This sex ratio can thus be written

$$r_{IJ,T} = \frac{1}{2} + \frac{1}{\sqrt{\pi}}\int_{0}^{\alpha_{IJ,T}/\sqrt{2}} e^{-\alpha^2}\,d\alpha \qquad (1)$$

where α represents the standardized amount of liability trait ($\alpha = \frac{A - \zeta}{\sigma_E}$). It can be easily checked from equation (1) that genotype *IJ* will produce both sexes in equal proportions at the temperature for which its

liability-trait value exactly matches the threshold (i.e., $A_{IJ,\tilde{T}} = \zeta$ and $\alpha_{IJ,\tilde{T}} = 0$), referred to as its pivotal temperature ($\tilde{T}_{IJ}$).

Parameter estimation

Equation (1) can be used to predict the sex ratio produced by a given genotype at a given temperature. Reciprocally, it can also be used to infer α_{IJT} values from observed sex ratios. For illustration (Fig. 2), we calculated α_{IJT} values at different temperatures for four TSD lineages, namely the fish *Odonthestes bonariensis* (Strussmann et al. 1997) and *Poeciliopsis lucida* (TSD strain; Sullivan & Schultz 1986), the lizard *Niveoscincus ocellatus* (Wapstra et al. 2009) and the turtle *Chrysemys picta* (Ewert, Echtberger, & Nelson 2004). The excellent fit supports the assumption of a single underlying genotype in all four cases (i.e., pure TSD). Reaction norms appear linear, with positive slopes in fish (β/σ_E = 0.51°C^{-1} and 0.28°C^{-1} respectively) and negative slopes in reptiles (β/σ_E =−0.40°C^{-1} and −1.38°C^{-1}). The pivotal temperatures (i.e., temperatures providing equal sex ratios), obtained as the intercept of the reaction norms with the abscissa ($\alpha_{IJ\tilde{T}}$=0), amount to 24.3°C, 25.3°C, 17.7°C and 26.9°C, respectively.

From the normal-distribution assumption, sex ratio is expected to be a sigmoid function of temperature in pure TSD systems such as in *O. bonariensis* (Fig. 3A). In heterogametic species with mixed sex determination, by contrast, the two genotypes are expected to generate a double sigmoid, as illustrated for two male-heterogametic species in Fig. 3B, C, namely the newt *Triturus cristatus* (Wallace, Badawy, & Wallace 1999) and the fish *Patagonina hatcheri* (Strussmann et al. 1997). In both cases, data were fitted assuming linear and parallel norms of reaction. In *P. hatcheri*, the *XX* and *XY* genotypes are more differentiated on the α axis (21.2, as opposed to 6.4 in *T.cristatus*) but display closer pivotal temperatures (25.1°C and 15.4°C respectively, as opposed to 31.2°C and 8.4°C for *T. cristatus*), because the slope (in terms of β/σ_E) is much steeper (2.17°C^{-1} vs. 0.28°C^{-1} for *T. cristatus*).

Intersex individuals sometimes appear around the pivotal temperature, due to disorders of sexual development arising when the liability-trait value lies too close to the threshold. Their frequency allows estimating an intersex range along the α axis. In the female-heterogametic salamander *Pleurodeles waltl*, for instance, the *ZW* genotype produces at 30°C 44% females, 46% males and 10% intersex individuals (Dournon, Houillon, & Pieau 1990, Table 3). Thus, assuming a normal distribution for the liability trait, intersex occurs in this species for α values ranging −0.13 to + 0.13.

EVOLUTIONARY RESPONSES TO TEMPERATURE CHANGE

All of the systems illustrated here are expected to show evolutionary response to temperature change, stemming, for example, from climatic change or range expansion. The short-term response will be a sex-ratio bias: for example, rearing medaka fish at 32°C will produce an excess of males in the first generation, because some *XX* individuals develop into males. Within a few generations, however, sex-ratio selection will restore a Fisherian sex ratio by lowering the frequency of the Y chromosome. In Appendix A, we derive the equilibrium frequency values for all three genotypes involved (*XX*, *XY*, and *YY)* and for the Y chromosome as a function of temperature and σ_E^2. Specific values are also derived at the several pivotal temperatures. In the following, we discuss in more intuitive terms the evolutionary outcomes predicted from consistent increases or decreases in temperature.

Temperature increase

Let us start with an initial situation (T= 0) where the liability-trait values for the *XX* and *XY* genotypes define a male-heterogametic system. Due to sex-ratio selection, the two genotypes are in equal proportions and Y frequency approximates 0.25 (Fig. 4A). As temperature increases, a few *XX* individuals turn into males, inducing a mixed system with genetic and environmental components. Sex-ratio selection will automatically lower the frequencies of *XY* individuals (and thus of Y) so as to maintain an even sex ratio at the population level. Note, however, that sex ratios are biased within families, because mating between *XY* males and *XX* females produces an excess of males, whereas mating between *XX* males and *XX* females produces an excess of females.

At the *XX* pivotal temperature (e.g., 25.1°C in *P. hatcheri* or 31.2°C in *T. cristatus*, Fig. 3), half of the *XX* individuals develop into males, so that within a few generations *Y* is lost and sex determination becomes purely environmental. As temperature further increases, the sex ratio becomes progressively male biased (Fig. 4A) and, were the process to continue, extinction would occur as soon as females become too rare to sustain a viable population. This sex-ratio bias will favor the emergence of a new sex-determination mechanism, selecting for any feminizing mutation, either on the same locus (*XW/XX*) or on any other locus involved in the sex-determining cascade (*XXfF/XXff*). The system will thus evolve toward female heterogamety.

Temperature decrease

If, from the initial conditions (T= 0), temperature is decreasing rather than increasing, then at some point a few *XY* genotypes will develop into females. Mating with *XY* males will produce 25% of *YY* progeny, which

may be viable or not, depending on the load of deleterious mutations that have accumulated on the nonrecombining segment of the Y chromosome.

In the case *YY* are viable and develop into fertile males, this genotype will increase in frequency, and *XX* females decrease in frequency, because *XY* genotypes progressively produce more females. At the pivotal *XY* temperature (e.g., 8.4°C in *T. cristatus*, Fig. 3B), *XX* and *YY* are produced in equal proportions, turning into females and males, respectively. For lower temperatures, *XY* genotypes develop more likely into females. Sex-ratio selection will thus increase the frequency of *YY* genotypes, and decrease that of *XX* genotypes. At mid distance between the *XY* and *YY* pivotal temperatures, the system will have reached a new state of pure GSD with female heterogamety. The genotypes *YY* and *XY* will produce males and females, respectively, in equal quantities, and the equilibrium frequency of Y tends toward 0.75 (eq. A3, Fig. 4A).

If temperature keeps on decreasing, the system will behave symmetrically to the high-temperature conditions. A pure ESD situation will be reached at the *YY* pivotal temperature, where allele Y will become fixed in the population (Fig. 4A). Thereafter sex ratio will progressively depart from even (Fig. 4A) so that the population will eventually go extinct due to absence of males, unless a masculinizing mutation, either on the same locus (*YY/YZ*) or on a different one (*YYmm/YYmM*), rescues the system (which will then turn back to male heterogamety).

Unviable *YY* genotypes will induce an earlier female bias (i.e., before the *XY* pivotal temperature is reached), and a strong segregation load (because crosses between *XY* males and *XY* females keep on producing 25% of lethal *YY* genotypes). Relative to the *YY*-viable situation, both *XY* and *XX* genotypes will reach higher frequencies (because the only males are *XY*). Extinctions are thus expected to occur earlier, unless the system is rescued by a masculinizing mutation (either *XX/XZ* or *XXmm/XXmM*).

Environmental variance effects

The equilibrium Y frequency is a stepwise function of temperature (Fig. 4A), decreasing from 1 at the *YY* pivotal temperatures to 0 at the *XX* pivotal temperature. Steps are strongly marked when the environmental component of phenotypic variance is low (i.e., the slope β/σ_E is steep) because the standardized liability-trait values lie then far apart from the threshold for most of the temperature ranges, so that sex reversal is rare. As a result, GSD systems (either male or female heterogamety) are stable over large ranges of temperatures, and show abrupt changes around critical temperature values. In contrast, a high environmental variance (i.e., shallow

β/σ_E slope) makes changes in equilibrium Y frequency smooth and continuous, resulting in a mixed sex-determination system over large temperature changes.

INDIVIDUAL-BASED SIMULATIONS

Rationale and settings

Whether temperature changes result in extinctions or turnovers will depend on drift and mutations, in addition to the selective pressures outlined here above. To cross-validate our analytical predictions and investigate the interplay between these evolutionary forces, we performed individual-based simulations, exploring a subregion of the domain of climatic change plotted in Figure 1.

Simulations (detailed description in Appendix B, see also Fig. S1) were run with a modified version of the program quantiNemo (Neuenschwander et al. 2008). Starting with a male heterogametic system (*XX/XY*) at T= 0, temperature was increased by progressive steps after a burn-in of 400 generations. We varied the environmental variance σ_E^2 (from 10^{-7} to 40) and the population size N (from 50 to 10,000) to investigate their effects on transition probabilities. A first set was run without any mutations to cross-validate our analytical expectations. In the other sets, we allowed mutations, either to a strongly feminizing allele W (set 2), or to randomly sampled allelic values from a large range of masculinizing and feminizing mutations (sets 3 and 4; further details in Appendix B). Mutations affected only the intercept (genetic up- or down-regulation of the liability trait), not the slope (assumed to reflect environmental effects stemming from physiological constraints), but we also performed simulations with a flat slope (β = 0) as a control for endothermy (body temperature assumed constant, independent of environmental change). We also ran simulations assuming *YY* lethality and/or some intersex sterility. Intersex sterility was implemented as a fitness decrease for genotypes with a liability-trait value close to the threshold (ranging −1.0 to 1.0 Appendix B, Fig. S2), and *YY* lethality by assigning a fitness of zero for *YY* genotypes. For each combination of parameter values, we ran 500 replicates over 3,000 generations.

Cross-validating analytical results

The results obtained from the first set match remarkably well our analytical expectations (see e.g., Fig. 4B for N= 1,000), notwithstanding slight departures stemming from genetic drift at small population sizes (N≤ 500) and large environmental variances (σ_E^2 > 5; data not shown). At starting conditions (T= 0), the Y frequency

slightly exceeds 0.25 for high σ_E^2 values (because the few sex-reversed *XY* females produce some *YY* males). As expected from the analytical calculations, Y was quickly lost (and ESD achieved) as soon as the *XX* pivotal temperature was reached. At higher temperatures, increases in the frequency of *XX* males could not be accommodated anymore by a decrease in Y frequency, so that male biases progressively accrued (Fig. 4B) and extinctions eventually occurred at low σ_E^2 values.

Both gene dynamics and extinction probabilities were affected by environmental variance σ_E^2 in interaction with population size. As expected from analytical results, a large environmental variance (shallow β/σ_E slope) increased the proportion of sex-reversed *XX* males below the pivotal temperature ($\alpha_{XX,T} < 0$), and thus lowered equilibrium Y frequency (Fig. 4). When genetic drift was large (i.e., small population size *N*), this selective pressure accelerated the loss of Y, and thus the transition to ESD. Above the pivotal temperature by contrast ($\alpha_{XX,T} > 0$), a large environmental variance σ_E^2 increased the proportion of females among *XX* individuals, and thereby moderated the biases in sex ratio (Fig. 4). Extinction risk (stemming from such biases) was thus large at small σ_E^2 (steep β/σ_E slope), but declined rapidly with increasing σ_E^2, and the more so in large populations.

Mutations and transitions to new GSD

The remaining sets of simulations allowed for environmental change to be combined with mutations to new alleles. These sets were used to investigate how sex-ratio selection interacts with drift and environmental variance in determining the timings and probabilities of transitions (or extinctions). Under the assumption of endothermy (β/σ_E = 0), the initial *XX/XY* system was usually maintained throughout (e.g., 63% to 75% of simulations at *N*= 500, depending on the mutation model). Multiallelic polymorphisms (XYW, akin to the system found e.g., in *Xiphophorus*, Orzack et al. 1980) sometimes occurred (20–35% of simulations at *N*= 500), while transitions to another recurrent pair were rare (2% to 5% at *N*= 500, depending on the mutation model).

Under the assumption of ectothermy ($\beta/\sigma_E \neq 0$), by contrast, the initial *XX/XY* system was always overturned. As all mutation models provided the same qualitative results, we present only the first one in the main text (set 2: mutation to one strongly feminizing allele W; Fig. 5) and the others as Supporting information (Fig. S3). The results can be classified in four qualitatively distinct processes, depending on whether the final outcome was extinction or transition to a new GSD, and whether this end result was preceded or not by an ESD period. (1) Transitions to ESD (by loss of Y) followed by extinction occurred when no feminizing allele was available during the ESD phase. (2) Transitions to ESD, followed by a new GSD (most often female heterogametic,

XW/XX) occurred when mutation to a feminizing allele occurred during the ESD phase. (3) Direct transition to a new GSD (most often female heterogametic, *XW/XX*), occurred when a feminizing allele appeared before the loss of Y, and was maintained throughout by chance to be then favored by sex-ratio selection. In outcomes (2) and (3), transition to a new male heterogametic GSD sometimes occurred (e.g., *WW*/*WY*), when Y was maintained at low frequency throughout, or a new masculinizing mutation arose. (4) In a few cases, finally, a transition to a new GSD was followed by extinction when the feminizing effect of the new allele was insufficient to cope with climatic change.

Outcome frequencies depended on population size *N* and environmental variance σ_E^2 (Figs. 5A and S3A). Direct transitions (outcome 3) were very rare in small populations, because feminizing mutations were both less likely to appear ($N\mu$ being up to 200 times smaller), and less likely to be maintained (due to strong drift). Environmental variance had no noticeable effect on direct transitions, but a strong effect on extinction rate (outcome 1), in interaction with population sizes: extinctions were very frequent at small *N* and low σ_E^2. Outcome 4 only occurred when both *N* and σ_E^2 were low (Fig. S3A).

Intersex sterility and YY lethality

Intersex sterility had a drastic influence on the outcomes, independent of whether *YY* was viable (Fig. 5B) or not (data not shown). ESD was rare and only observed for low population size and high σ_E^2 (shallow β/σ_E slope), whereas direct transitions to a new GSD (always male heterogametic, *WW*/*WY*) were frequent outcomes. Outcome 1 (ESD then extinction) was rare and outcome 4 (new GSD followed by extinction) occurred at low population size and low σ_E^2. Surprisingly, intersex sterility generally lowered extinction risks in small populations.

By contrast, *YY* lethality only had a slight negative effect on the probability of direct transitions. This was due to selection against the *WY/YY* recurrent pair, which prevented the feminizing allele W to spread before attainment of ESD (data not shown). More serious effects are obviously expected under temperature decrease (see above).

DISCUSSION

Sex-determination in a quantitative-genetics framework

The conventional definition of sex-determination systems (e.g., Valenzuela et al. 2003; Ospina-Álvarez & Piferrer 2008) is undoubtedly applicable in the extreme cases of pure GSD or pure ESD, when all the phenotypic variance of the liability trait in a population is either genetic or environmental: If for example, all individuals in a population share the same genotype, the reason why a focal individual develops into a male or a female can easily be assigned to its environment. In intermediate cases, however, such a dichotomous assignment is not possible, because genetic and environmental effects cannot be partitioned at the individual level: the liability-trait value necessarily depends on the interplay between genes and environment.

What can be partitioned, however, is the amount of variance in a population. The relevant question thus becomes that of the apportionment of phenotypic-sex variance into genetic and environmental factors. We think the alternative definition of sex-determination systems we propose here, and its formalization in a quantitative-genetics framework, offer better opportunities to account not only for intermediate situations, but also for transitions between sex-determination systems. Both seem common in cold-blooded vertebrates. The dynamics and diversity of sex-determination systems observed in nature, including transitions (e.g., Quinn et al. 2007), mixed systems (e.g., Quinn et al. 2007), or multiallelic polymorphisms (where X, Y, and W coexist, as observed in some fish and amphibians; Orzack et al. 1980; Miura et al. 1998), were actually regular outcomes in our simulations.

Why are transitions more frequent in cold-blooded vertebrates?

Several mechanisms have already been proposed to explain turnovers. From these models, new sex determiners may spread and invade if (1) some of the new sex genotypes have a higher intrinsic adaptive value (Bull & Charnov 1977; Orzack et al. 1980; Basolo 2001; Kraak & Pen 2002), (2) changes are driven by a genetic conflict arising from sex-chromosome meiotic drive or cytoplasmic sex-ratio distorters (e.g., *Wolbachia*, (Hamilton 1967; Werren & Beukeboom 1998; Caubet et al. 2000), (3) sex-ratio selection is affected by changes in the production costs of male and female offspring (Kozielska et al. 2006), or (4) the new sex determiners are linked to a locus with strong sex-antagonistic effect (van Doorn & Kirkpatrick 2007). None of these mechanisms, however, seem to specifically apply to cold-blooded vertebrates, and hence to account for the striking contrast offered with birds or mammals.

From our formalization, turnovers in cold-blooded vertebrates are the mere consequences of environmental changes. The underlying rationale is simple: Because temperature affects sex determination, environmental changes will induce sex-ratio biases, which in turn should favor the emergence of new sex-determination genes or alleles. Sex-ratio selection is a powerful force, already invoked to explain turnovers between GSD systems (e.g., Caubet et al. 2000; Kozielska et al. 2006) or transitions from GSD to TSD (e.g., Bull 1981). As our results show, sex-ratio selection is triggered in ectotherms by environmental changes, due to the thermodynamic constraints shaping their reaction norms, and is thereby expected to induce turnovers in sex-determination systems.

This process does not affect warm-blooded vertebrates, because thermoregulation prevents the expression of temperature effects. Besides birds and mammals, some fish (e.g., tunas) do also show some levels of endothermy, but usually no parental thermoregulation during the thermally sensitive window of embryonic development. Carcharinid sharks apparently possess the relevant combination of viviparity and endothermy, but sex-determination mechanisms are unfortunately poorly known in Elasmobranchs (although many seem to display heteromorphic sex-chromosomes; Maddock & Schwartz 1996).

Our model directly relates the prevalence of homomorphic sex chromosomes in cold-blooded vertebrates to the temperature sensitivity of sex determination imposed by ectothermy. The driving forces behind turnovers are to be found in climatic changes or range expansions. Note that the present argument differs from Perrin (2009), who also proposes a link between homomorphic sex chromosomes and the sex-reversal events induced by temperature changes, but without any turnover. From his model, X–Y recombination may occur in sex-reversed *XY* females (because recombination depends on phenotypic sex, not on genotypic sex), and this should prevent the decay of Y chromosomes even in absence of turnovers. As turnovers do occur in cold-blooded vertebrates (e.g., Hillis & Green 1990; Ezaz, Stiglec, et al. 2006a; Volff et al. 2007), our model provides a (nonexclusive) alternative to Perrin (2009) to explain the prevalence of homomorphic sex chromosomes in these groups.

Conservation issues

Whether the processes underlying sex-determination are dichotomous or continuous is not only a semantic question. It will affect the dynamics of turnovers, but also the risks of extinction. It was recently argued that TSD species are put at significant extinction risk by climatic changes, due to the progressive biases in sex ratios induced by temperature rises (e.g., Janzen 1994; Janzen & Morjan 2001; Mitchell et al. 2008). GSD species are often implicitly considered as protected against such changes, because genotypic systems (e.g., *XY* or *ZW*) are

expected to necessarily produce even sex ratios. From our formalization, however, environmental change will also induce sex-ratio biases in such systems. Mass events of sex reversal and sex ratio biases have already been documented in natural populations of amphibians and fish supposed to display pure GSD (e.g., Nagler et al. 2001; Matsuba et al. 2008).

From our simulations, extinctions are possible outcomes from such events. Small populations, in particular, were often locked in one recurrent pair or in ESD, which prevented adaptive transitions during climatic changes, and induced strong genetic loads associated with *YY* lethality, intersex sterility, or biased sex ratios. This often led to extinction when combined with low environmental variance in the liability trait *A* (steep β/σ_E slope). Further negative effects of small population size stemmed from the lower probability of appearance of a new mutation, and a higher demographic stochasticity in sex ratios, inducing a risk of loosing by chance all members of one sex. These processes are also likely to hinder range expansions, because colonizing populations reaching new (and climatically different) areas often stem from rare long-distance propagules and have small effective population sizes.

The environmental component of variance (σ_E^2) also mattered, with overall positive effects. High values (shallow β/σ_E slope) made any genotype more likely to produce individuals of both sexes, which provided insurance against extinction, particularly under ESD. Interactions with drift were important, as in many instances large σ^2_E values could counteract the negative effects of small population sizes. As a consequence, extinctions are expected to occur mostly when both *N* and σ_E^2 are low.

YY lethality and intersex sterility

From our preliminary results, *YY* lethality limits the evolutionary potential of populations and enhances the risk of extinctions. This obviously prevents the evolution toward some specific systems (e.g., *XY*/*YY*, otherwise favored at low temperature), but also imposes segregation loads when *YY* genotypes are not directly involved in a recurrent pair (e.g., *XX/XY* or *WW/WY*), because sex reversal induced by high σ_E^2 values produces unfit *YY* individuals. This may favor the transition to alternative recurrent pairs (e.g., *XW/XX*), but will also induce extinctions in small populations with reduced adaptive potential. *YY* lethality is likely to emerge during periods of evolutionary stasis, as functional genes become progressively involved into the expanding nonrecombining segment and accumulate deleterious mutations (Ohno 1967). Frequent turnovers may thus allow maintaining the evolutionary potential and adaptability of populations, a point deserving further investigations.

Intersex sterility also had drastic influences on evolutionary paths, selecting for genotypes with liability-trait values far apart from the threshold. This might actually explain why sex reversal in cold-blooded vertebrates usually occurs outside the natural range of environmental conditions. In our simulations, this selective pressure often prevented the evolution of ESD (because *XX* genotypes were strongly counter-selected when producing sex-factor values within the intersex range) and favored direct transitions to a new male-heterogametic GSD system (e.g., *WW/WY*, with strongly masculinizing and feminizing effects of Y and W, respectively). It is worth noting that the increased selection for a feminizing allele W prevented its loss by drift in small populations, thereby lowering their extinction risk. This opposed our intuitive expectation that intersex sterility would increase extinction risks.

Empirical issues and model extensions

The quantitative predictions stemming from our simulations obviously depend on specific model assumptions and parameter values. As underlined above, empirical data can be used to calibrate key parameter values in specific cases. Values for α_{IJT} (standardized liability-trait value for genotype *IJ* at temperature *T*) can be estimated from the proportion of sex reversals at this temperature. The shapes of reaction norms (and evidence for mixed systems) can be obtained by plotting α values as a function of temperature (Fig. 2). From our few empirical examples, parameter estimates fall well within the range used for simulations; empirical estimates of $|\beta/\sigma_E|$, for instance, lie between 0.28 and 2.2 (Figs. 2 and 3). Our assumptions of linear reaction norms and normal distribution of environmental variance are also well supported in the examples provided. Note however that a similar formalization might be provided for curvilinear reaction norms, such as found in lizards and turtles where males are only produced at intermediate temperatures (Ewert et al. 2004; Quinn et al. 2007). A larger scale literature survey to estimate the range of shapes and parameter values for reaction norms along the lines presented here (together with other relevant features such as intersex sterility or *YY* lethality) would constitute welcome empirical extensions of the present work.

Regarding theoretical extensions, the interaction between turnovers and Y decay will also constitute an important avenue for future research. Our preliminary results suggest that frequent turnovers might allow maintaining the evolutionary potential of populations, but a detailed formalization is required to precisely account for the dynamics of deleterious mutations. Sex-antagonistic genes are bound to interfere with this process, being the ultimate cause for the evolution of nonrecombination (e.g., Bergero & Charlesworth 2009). The fixation of male-beneficial sex-antagonistic alleles on the Y chromosome is expected to counter-select sex-reversed *XY* females, and thereby to affect the dynamics of turnovers.

Finally, a similar approach might be used to address the evolution of TSD as an adaptive strategy (e.g., Janzen & Phillips 2006). In our model, TSD only occurred as a side result when homozygous genotypes reached their pivotal temperature, and was counter-selected by sex-ratio selection at other temperatures. Evolving TSD as an adaptive strategy clearly requires behavioral adaptations allowing to fine-tune embryonic temperature so as to produce desired sex ratios. TSD might then outcompete GSD when optimal sex ratios differ from even (Hamilton 1967; Freedberg & Taylor 2007), or when temperature also affects fitness in a sex-specific way (Charnov & Bull 1977; Bull 1981; Bulmer & Bull 1982; Conover 1984). Intersex sterility is also bound to play a crucial role in this context, because genotypes are mostly affected close to their pivotal temperature (Bull 1981). This should favor the evolution of reaction norms with extreme sensitivity to temperature (steep β/σ_E slope) across the intersex sterility domain. Detailed investigations along the lines sketched out in the present article might help shedding light on this complex and fascinating issue.

ACKNOWLEDGMENTS

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FIGURES

Figure 1: Quantitative-genetics model of sex determination with gene–environment interactions. The liability-trait value $A_{IJ,T}$ produced by a genotype IJ depends on temperature T (norms of reaction are assumed here linear and parallel with slope β). Individuals develop into males if $A_{IJ,T}$ exceeds a threshold value (here arbitrarily fixed to 0), and into females otherwise. The among- individual variance in microhabitats within populations (Gaussian curves on the T axis) translates into an environmental variance around genotypic means (Gaussian curves on the A axis, with standard deviation σ_E). At initial conditions (T = 0), genotypic values define a male-heterogametic system ($A_{XX,0} < 0$ and $A_{XY,0} > 0$). At the XX pivotal temperature ($\tilde{T}_{XX}$), the system is purely TSD ($A_{XX,\tilde{T}} = 0$) with even sex ratio. At higher temperatures, sex-ratio selection will favor a feminizing mutation (W), leading to a female-heterogametic system ($A_{XW,T} < 0$ and $A_{XX,T} > 0$). Similar transitions are expected for temperature decreases.

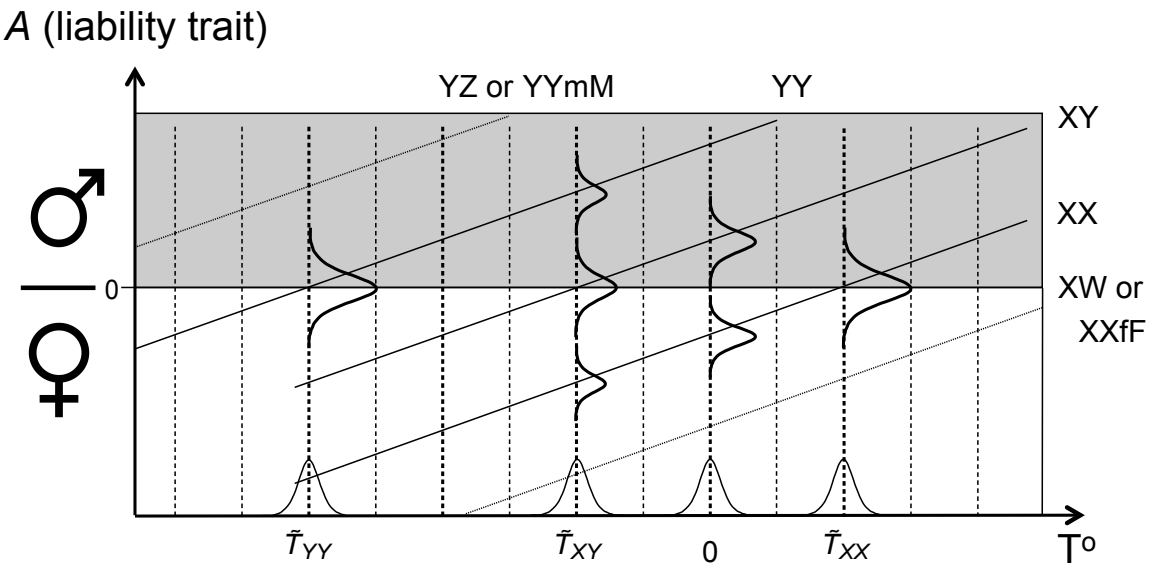


Figure 2. Standardized liability-trait values (α) as a function of temperature for the two fish *Odonthestes bonariensis* (A) and *Poeciliopsis lucida* (B), the lizard *Niveoscincus ocellatus* (C) and the turtle *Chrysemys picta* (D). In all four cases, the good fit supports the assumption of a single genotype (pure TSD) with a linear norm of reaction. Data are from Strussmann et al. (1997), Sullivan and Schultz (1986), Wapstra et al. (2009) and Ewert et al. (2004). For details see Appendix C.

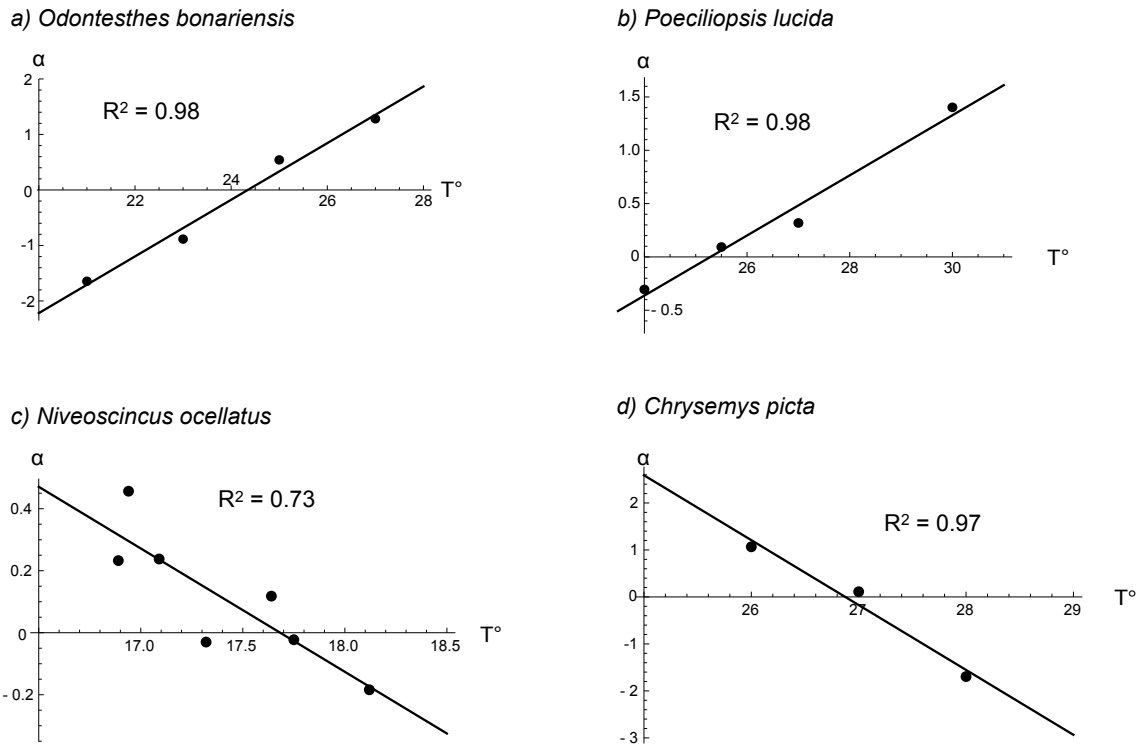


Figure 3. Sex ratio (*r*) is a sigmoid function of temperature in a pure TSD system (*Odonthestes bonariensis*, (A) and a double-sigmoid function in mixed systems (here with male heterogamety; *Triturus cristatus*, [B] and *Patagonina hatcheri*, [C]). Norms of reaction are assumed linear and parallel, and environmental variance normally distributed. Horizontal dashed lines plot the expected sex ratios at pivotal temperatures ($\tilde{T}_{XX}$ and $\tilde{T}_{XY}$, arrows), namely *r*= 0.5 (at $\tilde{T}_{XX}$) for pure TSD, and *r*= 0.75 (at $\tilde{T}_{XX}$) or 0.25 (at $\tilde{T}_{XY}$) for male heterogametic systems. Data are from Strussmann et al. (1997), Wallace et al. (1999) and Ospina-Álvarez et al. (2008). For details see Appendix C.

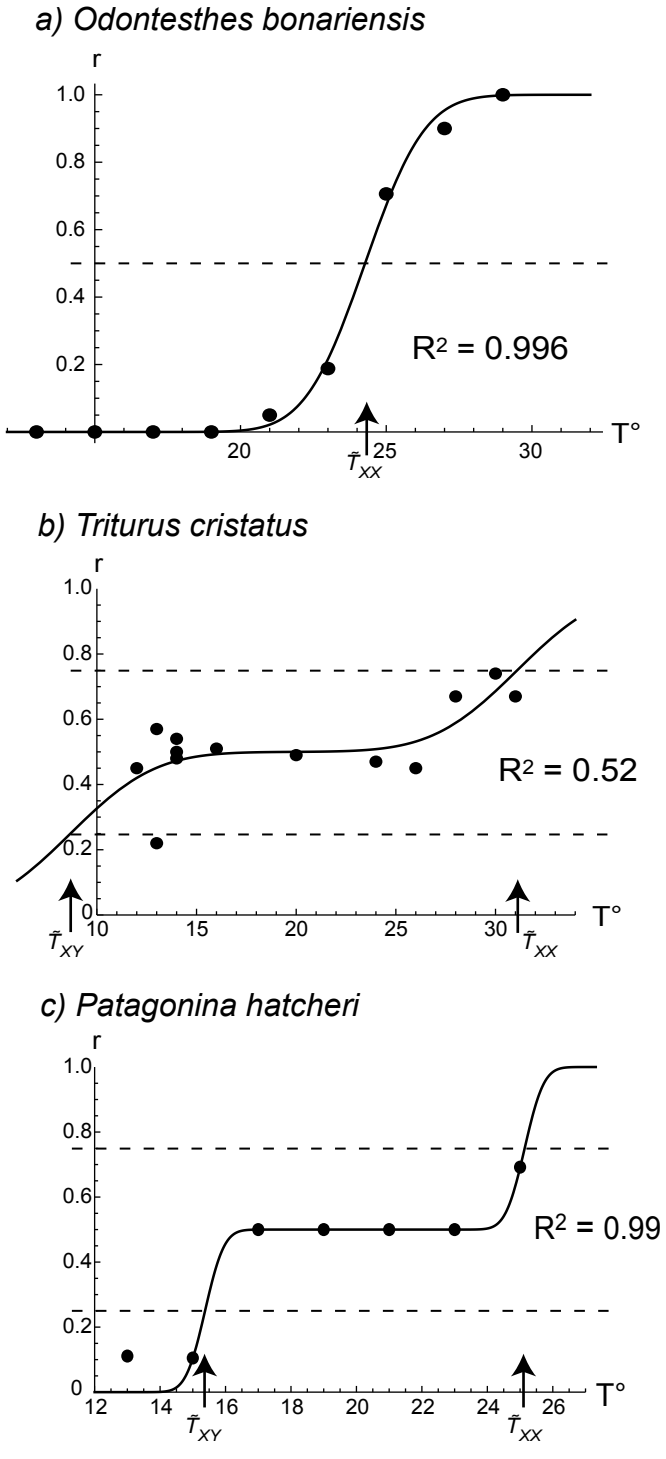


Figure 4. (A) The equilibrium frequency of Y (black lines, calculated from equation A3) drops from 1 to 0 as temperature T increases from the *YY*- to the *XX* pivotal temperature ($\tilde{T}_{YY}$ and $\tilde{T}_{XX}$ respectively). Changes are stepwise with marked steps for small environmental components of phenotypic variance (σ_E^2 = 0.5, plain line), and smoother steps for larger values (σ_E^2 = 2, dashed line; σ_E^2 = 8, dotted line). Outside this temperature range, sex ratio departs from even (gray lines, calculated from eq. 1). (B) Upper temperature range of Figure 4A with equilibrium values for Y frequencies (black) and sex ratios (gray). The simulation results (average over 500 simulations and 95% confidence intervals, *N*= 1000) are superimposed on predicted values for σ_E^2 = 0 .5 (squares, plain lines), 2 (triangles, dashed lines), and 8 (circles, dotted lines).

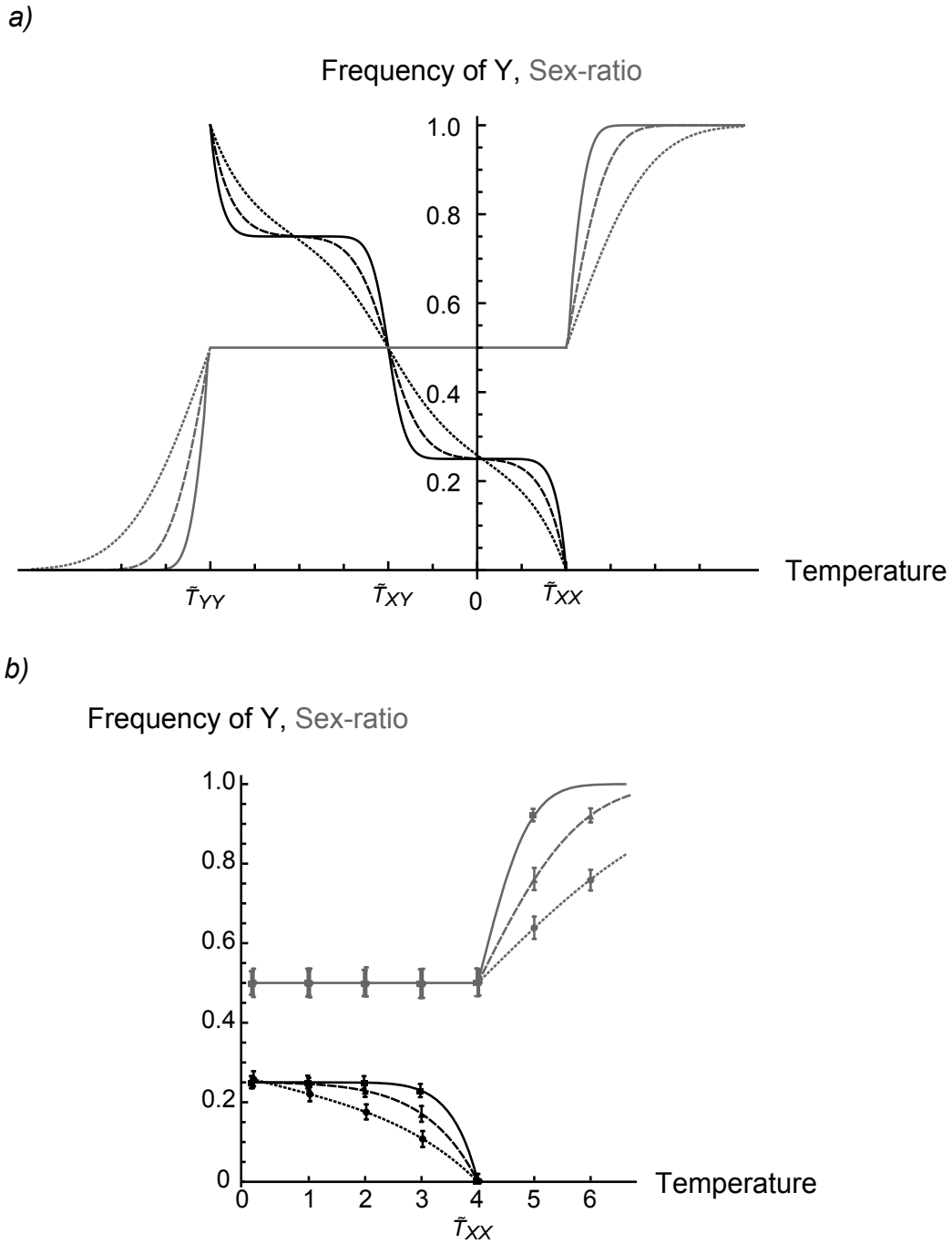
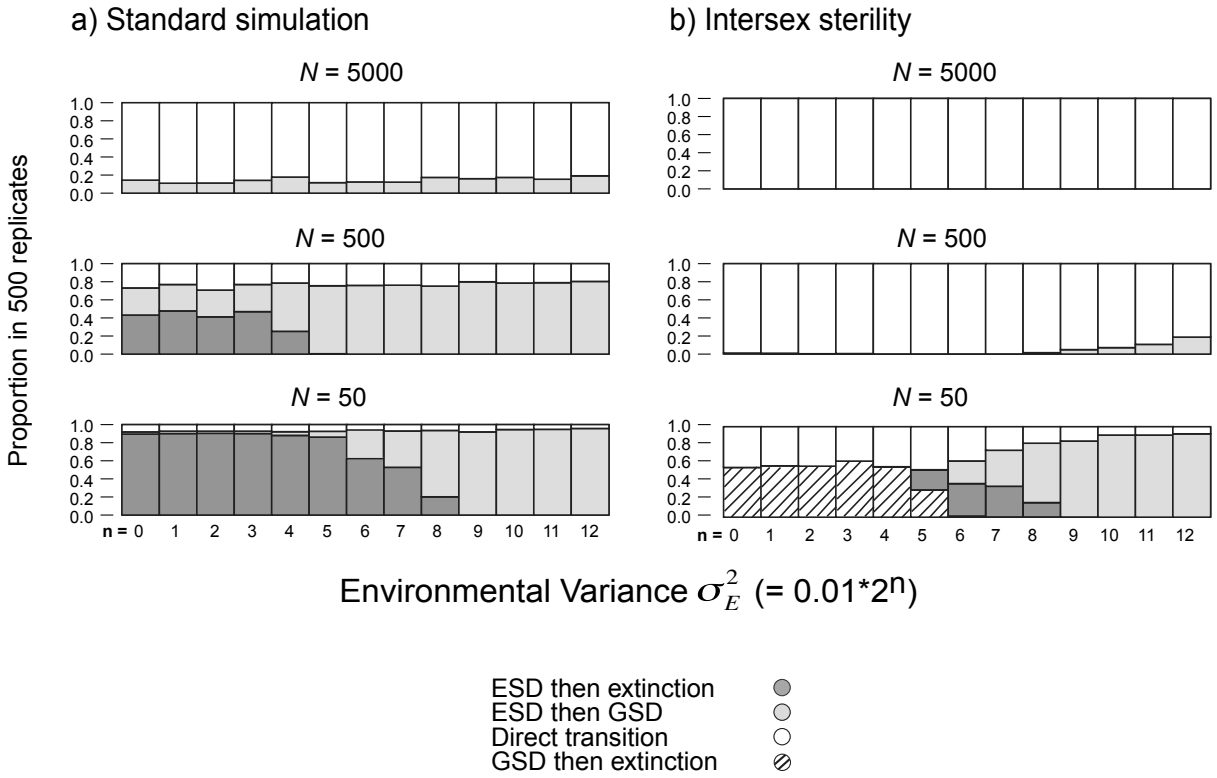


Figure 5. (A) Outcomes of simulations with mutations to a strongly feminizing allele W, for three different population sizes (N= 50, 500, 5000) as a function of environmental variance (logarithmic scale, σ_E^2 = 0.01 × 2n; min; max = 0.01; 40.96). Shown are the percentages of different outcomes over 500 simulations: dark gray bars, transition to ESD (*XX*), then extinction; pale gray bars, transition to ESD (*XX*) then to a new GSD (most often *XW/XX*); white bars, direct transition to a new GSD (most often *XW/XX*); shaded bars, direct extinction from a GSD. Extinctions only occurred at small population size and small σ^2_E. Transitions were often direct at large population sizes, independent of σ^2_E. (B) Same simulation settings with intersex sterility. Transitions were more often direct, and extinctions were less frequent, usually without an ESD phase.



TABLES

Table 1: Parameters and symbols of the model

$A_{IJ,T}$	Liability-trait value for genotype *IJ* (I,J=X,Y) at temperature T
ζ	threshold value for A, such that individuals develop into males for A> ζ and into females otherwise
σ^2_E	environmental component of the phenotypic variance in A within populations
$\alpha_{IJ,T}=(A_{IJ,T}-\zeta)/\sigma_E$	standardized liability-trait value for genotype *IJ* at temperature T
$r_{IJ,T}$	proportion of males produced by genotype *IJ* at temperature T
$R_{IJ,T}=r_{IJ,T}/(1-r_{IJ,T})$	number of males per female for genotype *IJ* at temperature T
$p_{IJ,k}$	frequency of individuals with genotype *IJ* and sex k (=m,f) within the population
β	slope of reaction norms (increase in the production of the liability factor A with a unit increase in temperature)
$\tilde{T}_{IJ}$	Pivotal temperature for genotype *IJ* (defined by $R_{IJ,\tilde{T}}=1$).

SUPPLEMENTARY FIGURES

Figure S1. Sub-region of Fig.1 explored through individual-based simulations, with reaction norms for all possible genotypes. The *YY* norm is dashed to point out *YY* lethality assumed in some simulations. Also indicated are the region of *A* values leading to intersex sterility (horizontal dotted lines), and the *XX* pivotal temperature ($\tilde{T}_{XX}$).

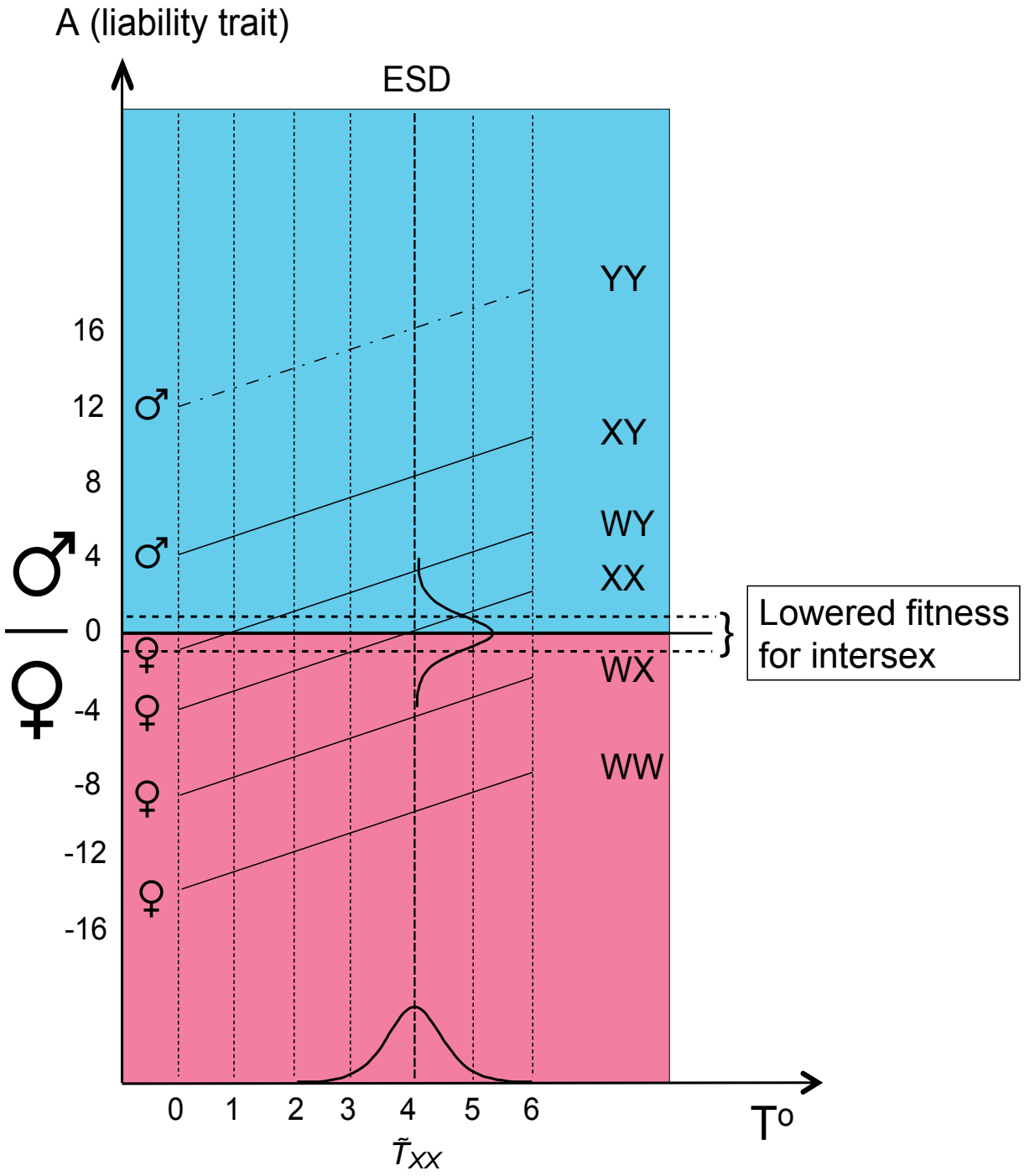


Figure S2. Intersex sterility model. Male and female fitness were modeled as logistic functions of the liability trait *A* (eq. B1). Male fitness (dashed blue line) is close to zero for *A* values below 0.5 (A_m , male inflexion point) and quickly reaches 1 for larger values, while female fitness (plain red line) quickly drops from one to zero for *A* values larger than -0.5 (A_f , female inflexion point).

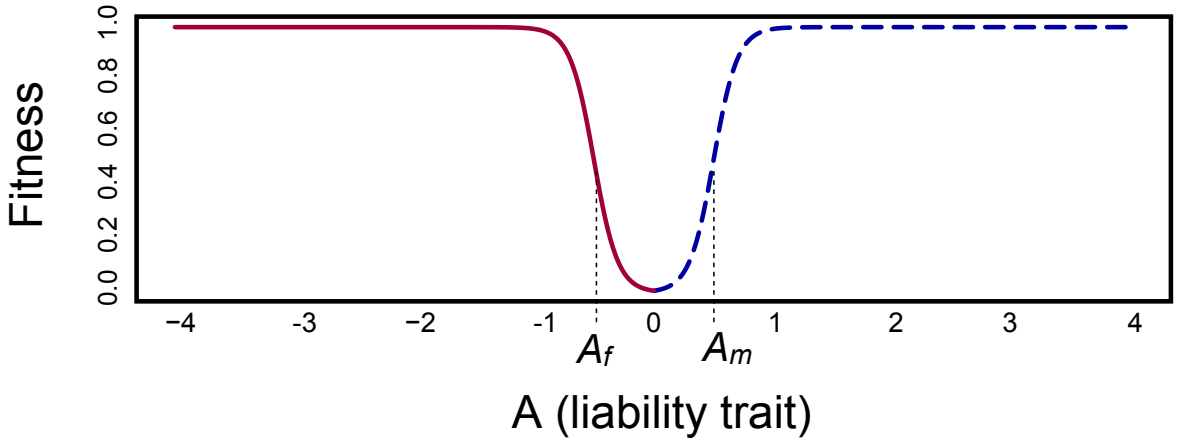
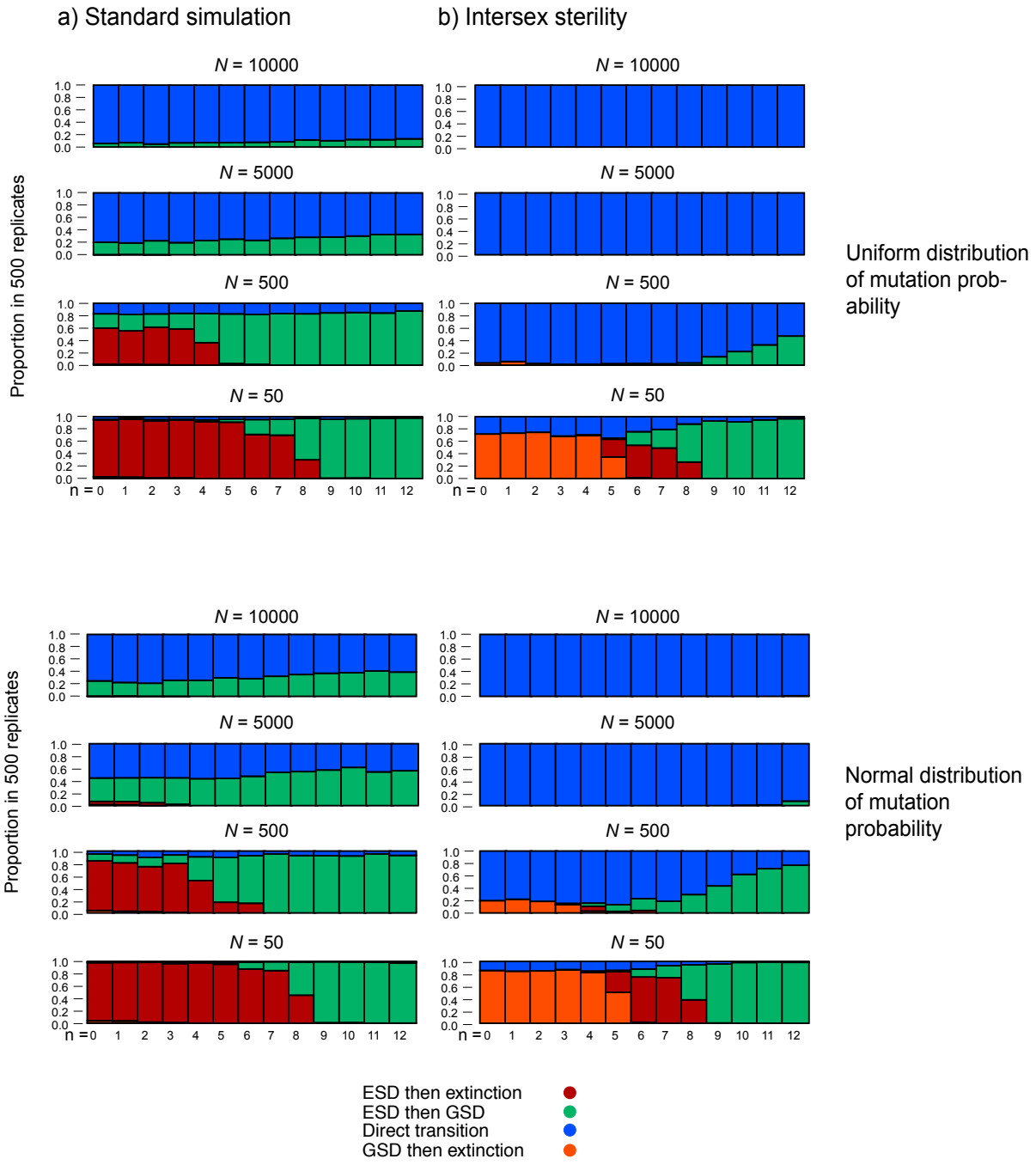


Figure S3. Outcomes of simulations with mutations to any of 161 possible masculinizing or feminizing alleles. Shown are the percentages over 500 simulations of four possible outcomes as a function of environmental variance (logarithmic scale: $\sigma_E^2 = 0.01 * 2^n$; min; max = 0.01; 40.96). Red bars, transition to ESD (*XX*) by loss of Y, then extinction; green bars, transition to ESD (*XX*) then to a new GSD (most often *XW/XX*); blue bars, direct transition to a new GSD (most often *XW/XX*); orange bars: direct extinction from a GSD. Distributions of mutation probabilities are either uniform (upper panels) or normal (lower panels), and simulations are run assuming intersex fertility (left panels) or sterility (right panels). Extinctions (red and orange) only occurred at low population sizes and small σ_E^2. Transitions were most often direct for large population size (blue), mostly independent of σ_E^2. Intersex sterility lowered occurrences of ESD and extinctions.



APPENDIX A: EQUILIBRIUM GENOTYPIC FREQUENCIES

The dynamics of genotypic frequencies are set by the recurrence equations:

$$p_{YY_{t+1}} = \left[\frac{p_{YYm_t} + p_{XYm_t}/2}{\sum_{I,J=X,Y} p_{IJm_t}}\right]\left[\frac{p_{YYf_t} + p_{XYf_t}/2}{\sum_{I,J=X,Y} p_{IJf_t}}\right], \quad \text{(A1a)}$$

$$p_{XX_{t+1}} = \left[\frac{p_{XXm_t} + p_{XYm_t}/2}{\sum_{I,J=X,Y} p_{IJm_t}}\right]\left[\frac{p_{XXf_t} + p_{XYf_t}/2}{\sum_{I,J=X,Y} p_{IJf_t}}\right], \quad \text{(A1b)}$$

and

$$p_{XY_{t+1}} = \frac{p_{YYm_t}\left[2p_{XXf_t} + p_{XYf_t}\right] + p_{XXm_t}\left[2p_{YYf_t} + p_{XYf_t}\right] + p_{XYm_t}\left[p_{XXf_t} + p_{XYf_t} + p_{YYf_t}\right]}{2\sum_{I,J=X,Y} p_{IJm_t} \sum_{I,J=X,Y} p_{IJf_t}} \quad \text{(A1c)}$$

where $p_{IJm} = p_{IJ}r_{IJ,T}$ and $p_{IJf} = p_{IJ}(1 - r_{IJ,T})$ represent the proportions of individuals within the population which have genotype *IJ* and are males (respectively females). Sex-ratio selection eliminates the X allele for temperatures below the *YY* pivotal temperature, so that sex determination becomes purely environmental. Similarly, for temperatures above the *XX* pivotal temperature (e.g. 25.1°C in *Patagonina hatcheri* or 31.2°C in *Triturus cristatus*, Fig.3), sex-ratio selection eliminates the Y allele, so that sex determination also becomes purely environmental. In between, sex-ratio selection adjusts X and Y frequencies so as to produce even sex ratios ($r = 0.5$), and the system is purely genotypic or mixed.

Genotypic equilibrium frequencies within this range can be found by solving the recurrence equations (A1), *i.e.* setting $p_{IJk_{t+1}} = p_{IJk_t}$ ($k = m, f$), while noting that, from sex-ratio selection, $\sum_{I,J=X,Y} p_{IJk} = 0.5$. Writing $R_{IJ} = \frac{r_{IJ}}{1 - r_{IJ}}$ the number of males per female for genotype *IJ,* the equilibrium frequencies for females are given by:

$$\hat{p}_{XXf} = \frac{1}{D}\left\{R_{XY}R_{YY}\left(1 + R_{XX}\right) - \frac{\left(R_{XX} + R_{YY}\right)\left(R_{YY} + R_{XY}^2\right) - \left|R_{YY} - R_{XY}\right|C}{2}\right\}, \quad \text{(A2a)}$$

$$\hat{p}_{XYf} = \frac{1}{2} + \frac{1}{D}\left\{R_{XX}R_{YY}\left(1 - 2R_{XY}\right) + R_{XY}\left(R_{XY} - 1\right)\left(R_{XX} + R_{YY}\right) + \frac{R_{XX}^2 + R_{YY}^2 - \left|R_{YY} - R_{XX}\right|C}{2}\right\}, \quad \text{(A2b)}$$

and

$$\hat{p}_{YYf} = \frac{1}{D}\left\{R_{XY}R_{XX}\left(1+R_{YY}\right) - \frac{\left(R_{XX}+R_{YY}\right)\left(R_{XX}+R_{XY}^2\right) - \left|R_{XY}-R_{XX}\right|C}{2}\right\}, \qquad \text{(A2c)}$$

where $C = \sqrt{\left(R_{XX}+R_{YY}\right)\left\{\left(R_{XX}+R_{YY}\right)\left(1+R_{XY}^2\right) - 2R_{XY}\left(1+R_{XX}R_{YY}\right)\right\}}$, and $D = R_{XY}\left(R_{XX}^2+R_{YY}^2\right) - 2\left(R_{XX}R_{YY}+R_{XY}^2\right)\left(R_{XX}+R_{YY}\right) + 6R_{XX}R_{XY}R_{YY}$. The corresponding equilibrium frequencies for males are given by $\hat{p}_{IJm} = \hat{p}_{IJf}R_{IJ}$, so that the equilibrium frequencies of X and Y become:

$$\hat{p}_Y = 1 - \hat{p}_X = \frac{\hat{p}_{XYf}}{2}(1+R_{XY}) + \hat{p}_{YYf}(1+R_{YY}). \qquad \text{(A3)}$$

This equilibrium frequency of Y is displayed in Figure 4a, together with sex ratios at equilibrium (eq 1), as a function of temperature *T* for different environmental variances.

Specific values can be calculated for different temperatures. At the *XX* pivotal temperature, R_{XX} = 1 by definition (i.e., *XX* individuals develop into males or females with the same probability). As R_{XY} and R_{YY} are very large (because most *XY* and *YY* individuals develop into males), *C* tends to $R_{XY}R_{YY}$ and *D* to $R_{XY}R_{YY}\left(R_{YY}-2R_{XY}\right)$, so that, for any $R_{YY} \geq R_{XY}$, both $\hat{p}_{XXf}$ and $\hat{p}_{XXm}$ tend to 0.5 (i.e., the only genotype left is *XX*).

At the *XY* pivotal temperature, R_{XY} = 1. As $R_{XX} = 1/R_{YY}$ are very small, *C* tends to $\sqrt{2}R_{YY}$ and *D* to R_{YY}^2, so that both $\hat{p}_{XYf}$ and $\hat{p}_{XYm}$ tend to $1-\frac{\sqrt{2}}{2}$, while both $\hat{p}_{XX}$ and $\hat{p}_{YY}$ tend to $\frac{\sqrt{2}-1}{2}$. It follows from (A3) that $\hat{p}_Y$ =0.5 (Fig. 4a).

At mid distance between the *XY* and *YY* pivotal temperatures, R_{YY} is very large, while R_{XY} and R_{XX} are very small (with $R_{XY} \geq R_{XX}$). *C* tends to R_{YY} and *D* to $R_{YY}^2\left(R_{XY}-2R_{XX}\right)$, so that $\hat{p}_{XXf} = \hat{p}_{XXm}$ vanish, and $\hat{p}_{YYm} = \hat{p}_{XYf}$ tend to 0.5, resulting in pure GSD with female heterogamety.

At the *YY* pivotal temperature, finally, R_{YY} = 1 while R_{XY} and R_{XX} tend to zero (with $R_{XY} \geq R_{XX}$). Hence *C* tends to 1 and *D* to $R_{XY}-2R_{XX}$, so that $\hat{p}_{YYm} = \hat{p}_{YYf}$ tends to 0.5. The system has thus fixed the *YY* genotype and reached pure TSD.

APPENDIX B: INDIVIDUAL BASED SIMULATIONS

Individual based simulations were run with a modified version of the program quantiNemo 1.0.3 (Neuenschwander et al. 2008), using a simple life cycle with non-overlapping generations (an inconsequential assumption because we look at equilibrium frequencies in stable environments, not at transient dynamics). Population size was kept constant (as long as both sexes were present). At reproduction a mother and a father was chosen randomly (with replacement) for each offspring (corresponding to a promiscuous mating system). In case of the intersex sterility scenario the random drawing of the parents depended on the fitness of the adults. Parental alleles at the sex-determining locus were randomly inherited.

Genotypic values and reaction norms

In order to start with symmetrical liability-trait values for males and females, allelic values for X and Y at initial conditions were arbitrarily set to -2 and +6 respectively with additive effects, so that *XX* genotypes developed into females ($A_{XX,T=0} = -4$) and *XY* genotypes into males ($A_{XY,T=0} = +4$). Norms of reaction were linear and parallel, with a slope β fixed to one (i.e., one unit increase in liability trait per unit change in temperature; Fig. S1). Note that the sensitivity of sex ratios to temperature is determined by β/σ_E , which was varied from 0.156 to > 3000 through our simulations.

Environmental change

Temperature was first maintained stable for 400 generations (which was largely sufficient for sex-ratio selection to equilibrate allelic frequencies), then raised by 6 degrees, in steps of one degree (i.e., one *A* unit) every 100 generations. During simulations, the sex ratio bumped at each climatic step, to be then quickly readjusted to 0.5 by a rapid decrease in the frequency of Y alleles. Corresponding equilibrium frequencies of Y were measured just before the next climatic step. At end conditions (*T*=6), genotypes were expected to produce only males in absence of environmental variance ($A_{XX,T=6} = +2$, $A_{XY,T=6} = +10$, $A_{YY,T=6} = +18$). Only *XX* individuals were expected to remain, with a sex ratio (proportion of males) equal to $\int_{A=0}^{\infty} N(2;\sigma_E)dA$.

Mutations

In a second set of simulation runs, we allowed mutation to a third, strongly feminizing allele (W) with initial allelic value -7. Mutations among the three allelic states occurred randomly at a fixed per-locus rate μ=10^{-4}. Note that the number of mutations occurring per generation in a population of size *N* is $2N\mu$, which was varied from 0.01 to 2 throughout our simulations. If *YY* individuals are viable, W and Y at initial conditions constitute an

alternative female-heterogametic recurrent pair (Fig. S1), with *YY* males $(A_{YY,T=0} = +12)$ and *YW* females $(A_{YW,T=0} = -1)$. At the end conditions, W reaches an allelic value of –4, again allowing two alternative recurrent pairs (Fig. S1), one female heterogametic $(A_{XW,T=6} = -3, A_{XX,T=6} = +2)$, and the other male heterogametic $(A_{WW,T=6} = -8$ and $A_{WY,T=6} = +5)$. During simulations, we assumed a recurrent pair to be "fixed" if the frequency of the alternative allele was below 5%. Similarly, we assumed ESD to be achieved when the frequency of the two alternative alleles was each below 5%.

We also ran simulations with 161 possible alleles at the sex-determining locus, with allelic values ranging from -16 to +16 (step 0.2). Two different mutation models were used, with distribution either uniform over the whole range (simulation set 3), or normally distributed around the threshold (with variance 7; simulation set 4).

Lethal YY and intersex sterility

The second set of simulations was also run assuming *YY* individuals to have zero fitness, and/or intersex individuals to be sterile. In the latter case, male and female fitness were modeled as logistic functions of the liability trait *A*:

$$w_{A,m} = \frac{1}{1+\exp\left(c\left(A_m - A\right)\right)} \qquad \text{(B1a)}$$

$$w_{A,f} = 1 - \frac{1}{1+\exp\left(c\left(A_f - A\right)\right)} \qquad \text{(B1b)}$$

where A_m and A_f are the inflexion points of the logistic curve (arbitrarily set to 0.5 and -0.5 respectively), and c defines the slope at this point (arbitrarily set to 10). Hence male fitness reached unity for *A* values above 1.0 and quickly dropped to 0 for lower values, while female fitness quickly dropped from one to zero for *A* values larger than -1.0 (Fig. S2).

APPENDIX C: MODEL FIT TO EMPIRICAL DATA

In the case of a single genotype (*XX*), the standardized liability-trait value ($\alpha_{XX,T}$) is directly calculated from r_T (population sex ratio at temperature *T*) using $r_T = \frac{1}{2}\left(1 + erf\left(\frac{\alpha_{XX,T}}{\sqrt{2}}\right)\right)$. If values are available for different temperatures, a regression of $\alpha_{XX,T}$ on *T* allows estimating the linear fit and calculating the regression coefficient β/σ_E (Fig 2).

Assuming two genotypes (e.g. *XX* and *XY*) at equal frequencies with parallel norms of reaction, the population sex-ratio at temperature *T* can be written:

$$r_T = \frac{1}{4}\left(2 + erf\left(\frac{\alpha_{XX,0} + T\,\beta/\sigma_E}{\sqrt{2}}\right) + erf\left(\frac{\alpha_{XY,0} + T\,\beta/\sigma_E}{\sqrt{2}}\right)\right), \qquad \text{(C1)}$$

which allows estimating the three parameters $\alpha_{XX,0}$, $\alpha_{XY,0}$ and β/σ_E through non-linear fitting (Fig.3).

CHAPTER II

THE BALANCED LETHAL SYSTEM IN CRESTED NEWTS: A GHOST OF SEX CHROMOSOMES PAST?

Christine Grossen, Samuel Neuenschwander and Nicolas Perrin

ABSTRACT

Crested newts and related species suffer from a balanced lethal system that makes 50% of offspring die early in development. All adults are heteromorphic for chromosome pair 1. The two variants (1A and 1B) have different deleterious alleles fixed on a non-recombining segment, so that heterozygotes are viable, while homozygotes are lethal. How can such a maladaptive trait appear and be maintained over evolutionary times in the face of natural selection? We propose a role for a sex-chromosome turnover from pair 1 (putative ancestral sex chromosome) to pair 4 (currently active sex chromosome), driven by a temperature shift (climatic changes or range expansion). Accordingly, 1A and 1B represent two variants (Y_A and Y_B) of the Y chromosome from an ancestral male-heterogametic system. We formalize this model through individual-based simulations, and show such a system to evolve with high likelihood, provided the masculinizing allele on chromosome 4 appeared after the elimination of the feminizing allele on chromosome 1.

INTRODUCTION

When a female crested newt lays a clutch, nothing will save her from loosing half of her investment. Fifty percent of all embryos will stop growing early in development, and die within a few days (Rusconi 1821; in Wallace 1987). Why is that so? Callan & Lloyd (1960) first noticed that chromosome pair 1 in *Triturus cristatus* adults was heteromorphic, and that the two variants (1A and 1B) harbored heterochromatic segments that did not form chiasmata in the oocyte lambrush bivalent stage. Further investigations showed that all non-viable offspring were homozygotes for one of the two variants (i.e. 1A/1A or 1B/1B). The same pattern was found to occur in the related *Triturus marmoratus*, but not in the more distant *Triturus alpestris* (Macgregor & Horner 1980). *T. marmoratus* and *T. cristatus* shared a common ancestor some 20 Million years ago (Arntzen, Themudo, & Wielstra 2007; Steinfartz et al. 2007). Experimental hybridization shows that chromosome 1A from one species, and 1B from the other, complement each other for larval viability (Sims et al. 1984). Hence, this balanced lethal system is likely to have evolved in their common ancestor.

How could this paradigm of dumb design have arisen and be maintained in the face of natural selection, which is expected to maximize individual fitness? Two main hypotheses have been proposed so far. The first one (Sims et al. 1984; reformulated by Sessions et al. 1988) postulates a "cytogenetic accident" (specifically, unequal genic exchange between the two homologous of an autosomal pair) that occurred in a common ancestor, making crossing over impossible in the concerned region. Following this arrest of recombination, several inversions, recessive lethal mutations and/or repeat sequences accumulated on the differential segment of chromosomes 1A and 1B. The question arises, however, how a mutation leading to such an extreme fitness reduction might get fixed in a population. The first offspring inheriting such an unequal genic exchange must have suffered from a drastically reduced fecundity and quickly be eliminated through competition with its sib.

The second hypothesis links the origin of the balanced lethal system to sex determination (Wallace 1984; 1987; Wallace et al. 1997; Wallace & Wallace 2000). Chromosome 1 was rapidly discarded as a candidate for sex determination, because heteromorphism was found to occur in both sexes (Morgan 1978; Macgregor & Horner 1980). The chromosome pair 4 was then identified as the sex chromosome pair, bearing a male heterogametic (*XX*/*XY*) system (Sims et al. 1984). Wallace (1987) suggested that 1A and 1B actually represent the two chromosomes of an ancestral *AA*/*AB* sex determination system. Accordingly, *BB* homozygotes are lethal because B accumulated deleterious mutations along its evolution in the heterogametic sex. This old system was then supplanted by the new *XY* system on chromosome 4, which operated effectively only in the former

heterogametic sex *AB*. It is not clear, however, why the *XY* system should only operate in an *AB* context, and how *AA* should become lethal, given that the *A* chromosome normally recombined in the former homogametic sex.

In the present paper we formalize an alternative hypothesis, which also relates this balanced lethal system to ancestral sex chromosomes. Specifically, we propose that the two homologs 1A and 1B represent two forms (respectively Y_A and Y_B) of the non-recombining sex chromosome from an ancient *XX*/*XY* system. We will first outline the main steps in the argument, then present modeling work that formalize it.

Non-recombining Y chromosomes necessarily accumulate deleterious recessive mutations, due to enhanced genetic drift, selective sweeps, background selection and Muller's ratchet (Charlesworth & Charlesworth 2000). Several Y haplotypes (i.e., fixed for different mutations) may segregate within populations. Such a situation has been well documented, for instance, in the guppy *Poecilia reticulata,* where at least three different Y variants have been shown to coexist in natural populations (Haskins et al. 1970 and references therein). These haplotypes code for different male coloration morphs, and are thus possibly maintained by frequency dependent selection occurring through female mate choice. When experimentally mating sex-reversed *XY* females with *XY* males from different haplotypes, 25% *YY* offspring are produced, which develop into fully viable and fertile males when heterozygous for the Y haplotypes, but are lethal when homozygotes (Haskins et al. 1970 and references therein). This necessarily implies that each haplotype has fixed one ore more recessive lethal mutations (e.g., loss of function of some house-keeping genes), and that different mutations occur in different haplotypes.

Sex reversal is easily triggered by temperature in many cold-blooded vertebrates, presumably due to thermal dependence in the expression of genes (or activity of enzymes) involved in the sex-determination cascade (see e.g. Grossen, Neuenschwander, & Perrin 2011). This is in particular true of crested newts, where high temperatures have a masculinizing effect, while low temperatures have a feminizing effect (Wallace et al. 1999; Wallace & Wallace 2000).

Let's thus assume that an ancestral newt population, harboring polymorphic Y haplotypes (Y_A and Y_B), experienced a feminizing temperature shift (due e.g. to climatic changes or range expansion), so that increasingly large numbers of XY_A or XY_B genotypes developed into females. When mating with normal XY_A or XY_B males, these females generated (among other offspring) lethal Y_AY_A and Y_BY_B homozygotes, as well as viable Y_AY_B heterozygotes. The balanced lethal system nowadays fixed in *T. cristatus* was thereby produced. For the very same reason (sex reversal), this temperature shift also generated biased sex ratios (namely, an excess of

females), thereby inducing a selective pressure for any masculinizing mutation able to restore even sex ratios (Grossen et al. 2011). As we will formalize below through individual-based simulations, this new mutation could spread to establish the new male-heterogametic system nowadays found on chromosome 4 in crested newt lineages, while still maintaining the Y_AY_B balanced lethal system trapped on the ancestral chromosomal pair 1.

METHODS

Conceptual Model

Sex-determination mechanisms can be modeled, in a quantitative genetics framework, as a continuum between purely genotypic processes (GSD) on the one hand, and purely environmental processes (e.g., temperature; TSD) on the other hand (Sarre et al. 2004, Grossen et al. 2011). Specifically, sex qualifies as a threshold trait, underlain by a liability factor (e.g., a sex hormone). Any individual will develop into a male if its liability trait value *A* exceeds the threshold (ζ), and into a female otherwise. This liability trait value $A_{IJ,T}$ depends on individual genotype *IJ*, on the mean local temperature *T*, and on individual deviation from this mean, stemming from micro-environment differences during the sensitive period of embryonic development. Hence, the phenotypic variance in the liability trait within populations has a genetic component (stemming from the coexistence of different genotypes) and an environmental component, assumed to be normal with mean 0 and standard deviation σ_E.

Genotypes are actually defined by reaction norms, representing the amount of the liability trait produced by this genotype as a function of temperature. Hence, depending on local temperature, a given genotype may develop in either male or female. Temperature shifts (due e.g. to climatic changes or range expansion) will thus generate biases in sex ratios, and thereby induce a selection for new sex-determination alleles or systems (see Grossen et al. 2011 for details, and e.g. Bulmer & Bull 1982; Quinn et al. 2007; Pen et al. 2010, for similar conceptualizations).

Implementation

Norms of reaction were assumed linear and parallel (a model with strong empirical support; Grossen & Perrin submitted), and thus modeled as $\alpha_{IJ,T} = \beta\left(T - \tilde{T}_{IJ}\right)$, where $\alpha_{IJ,T} = \frac{A_{IJ,T} - \zeta}{\sigma_E}$ is the standardized liability trait value for genotype *IJ*, β the standardized slope (change in standardized liability trait per unit change in

temperature, here arbitrarily fixed to 1), and $\tilde{T}_{IJ}$ the pivotal temperature for genotype *IJ* (i.e., the temperature at which this genotype produces males and females in equal proportions.

Sex genotypes were actually defined at two unlinked loci. The initial sex-determining locus (on chromosome 1) had one feminizing allele X and two masculinizing alleles Y_A and Y_B. The threshold ζ was arbitrarily set to 0, and allelic values at initial temperature conditions (T=0) were fixed to -1 for X and +3 for both Y_A and Y_B. Effects were additive, so that *XX* (genotypic value -2) developed into females, while XY_A and XY_B (genotypic values +2) developed into males. The second locus (on chromosome 4) was initially fixed for allele *m* (allelic value 0), but allowed to mutate to a masculinizing state *M* (allelic value +4).

We assumed simple life cycles with non-overlapping generations and constant population sizes. Reproduction occurred by choosing randomly, for each offspring, one father and one mother from the parental generation with replacement (which amounts to a promiscuous mating systems) and reiterating this process until reaching the carrying capacity.

Simulations

Simulations were run with a modified version of quantiNemo 1.0.3. (Neuenschwander et al. 2008). After a burn-in of 400 generations at $T°$=0, temperature was decreased to a final value of $T°$= - 8, reached after 1200 generations, by steps of one temperature unit every 100 generations (standard, Figure 1) or 0.1 every 10 generations (smooth). The smooth temperature change led to the same outcome as the standard change (data not shown).

At initial conditions ($T°$=0), alleles X, Y_A and Y_B were segregating on chromosome 1, while m was fixed on chromosome 4. In a first set of simulations, this locus was kept fixed to m (no mutation to *M* allowed), in order to investigate the evolution of the system under climatic change in absence of turnover. In a second set, we allowed masculinization mutations to occur (at rate 10^{-4} or 10^{-5}) right from the beginning. In the third set, this masculinizing mutation was only allowed after the climatic transition had occurred (from generation 3000, μ=10^{-4}).

For each set of simulations, we tested different carrying capacities (N = 50, 100, 500, 1'000, 5'000 and 10'000) and environmental variances (σ_E^2 from 0.3 to 4.2, steps 0.3). We also tested more extreme values of (σ_E^2 from 10^{-7} to 40.96), which did not change the picture (data not shown).

RESULTS

1. No masculinizing mutation

At initial conditions ($T°$ = 0), females were *mm XX,* males *mm* XY_A or *mm* XY_B, sex ratios were equal, and sex reversal absent (except for large environmental variance). A first temperature drop ($T°$=-2; Fig. 1) generated sex-reversed *mm* XY_A and *mm* XY_B females, which produced 25% viable sons (*mm* Y_AY_B) when mating with *mm* XY_B or *mm* XY_A males respectively, and 25% lethal sons (*mm* Y_AY_A or *mm* Y_BY_B) when mating with *mm* XY_A or *mm* XY_B males respectively. After a next drop ($T°$=-4), *mm* XY_A and *mm* XY_B genotypes mostly developed into females, while most adult males were *mm* Y_AY_B. Hence, 25% of offspring died (being either *mm* Y_AY_A or *mm* Y_BY_B depending on whether males mated with a *mm* XY_A or a *mm* XY_B female).

With a further temperature drop ($T°$=-6), half of the *mm* Y_AY_B genotypes developed into females, producing 50% lethal offspring when mated with *mm* Y_AY_B males. This system evolved towards pure TSD when *X* got lost (which often occurred by drift in small populations). However, *X* had a chance to survive at large σ_E^2 values, because a few *mm XY* then developed in males, with higher fitness than *mm YY* males. Finally, following the last temperature drop ($T°$=-8), *mm* Y_AY_B developed preferentially in females. This induced large female biases in sex ratios, leading to extinctions at small *N* and/or σ_E^2 values (red bars in Fig. 2A). Such extinctions did not occur when large *N* and/or σ_E^2 warranted the presence of at least a few males to rescue the population. This resulted in pure TSD (with 50% offspring mortality and strongly female-biased sex ratios) in the case X had been lost (blue bars), and a mixed system when large *N* and/or σ_E^2 allowed X survival (yellow bars).

2. Early masculinizing mutation

When the new masculinizing mutation *M* appeared early in the simulations (before X had any chance to be lost), it progressively increased in frequency as temperature dropped, to be finally fixed in the population. At $T°$=-4, this mutation first allowed evolution towards an alternative female heterogametic system (*mM XX* females and *MM XX* males, Fig. 1) with the potential to entirely loose Y_A and/or Y_B. At T°=-6, *MM XX* homozygotes produced males and females in equal quantities, allowing pure TSD to evolve, with the concomitant risk of loosing Y_A and/or Y_B as well. Hence, X was often fixed by drift in small populations. Lower temperatures ($T°$=-8) then restored selection in favor of Y_A and/or Y_B, (because *MM XX* increasingly developed into females), but mostly so at small environmental variance: High σ_E^2 values increased the probability that a few *MM XX* develop into males (fitter than *MM* XY_A or *MM* XY_B males, who produced lethal *MM YY* offspring when mating with *MM XY* females), and thus increased the risk of loosing Y_A and/or Y_B. The fixation of *X* (at small *N* or large σ_E^2) later

caused sex-ratio problems after the final temperature drop (T°=-8) because the only genotype left (*MM XX*) then mostly produced females. As a result, populations having fixed X went extinct (Fig. 2B; red bars), or survived under TSD with biased sex ratios (Fig. 2B; dark green bars). By contrast, populations having maintained Y_A and/or Y_B (large N and small σ_E^2; light green) could restore the initial male-heterogametic system on chromosome 1, with the masculinizing factor M fixed on chromosome 4 (i.e., *MM XX* females and *MM XY* males) and no sex-ratio biases.

3. Late masculinizing mutation

The end patterns in this case (Fig. 2C) also show three domains, the boundaries of which follow those from the first set (no masculinizing mutation; Fig 2A). The extinction domain was similar (red bars in both figures), but the other two domains presented different equilibrium SD systems. Whenever X could be maintained in absence of M (large N, large σ_E^2; yellow bars in 2A), the initial male-heterogametic system on chromosome 1 was restored after fixation of M (light green in Fig. 2C). By contrast, in all cases where X had been eliminated in absence of M (Fig. 2A; blue bars) the lethal system became fixed on chromosome 1 (with 50% offspring mortality), and sex was determined by a new male-heterogametic system on chromosome 4 ($mM\ Y_AY_B$ males and $mm\ Y_AY_B$ females; Fig. 2C blue bars), akin to the situation observed nowadays.

DISCUSSION

Examples of naturally occurring balanced lethal systems are quite rare (e.g. Tribolium castaneum (Dawson 1967) and Oenothera (Cleland 1972). An interesting situation occurs in the mole vole *Ellobius lutescens*, which also involves sex chromosomes. The species displays an uneven number of chromosomes (2n=17), both sexes being X0 (Lyapunova & Vorontsov 1975; Fredge 1994). Hence embryos are 25% XX, 25% 00, and 50% X0, of which only the latters develop. Whatever its evolutionary causes, this system is however less costly than the one under study, because embryonic mortality occurs well before the female has completed her reproductive investment. In *T. cristatus*, by contrast, the full investment is wasted.

Some amphibians are known to sacrifice some of their potential fertility as a part of their reproductive strategy. In the Strawberry poison frog *Oophaga pumilio* (= *Dendrobates pumilio*), for instance, 12% only of the eggs laid by a female are fertilized and develop into tadpoles. The other eggs remain unfertilized, and are used to feed developing larvae (Weygoldt 1980; Brust 1993). However, specific maternal oviposition strategies make sure that this investment will not benefit to non-kin. In the case of *T. cristatus*, many unrelated females

congregate to the same ponds to lay eggs, which makes highly unlikely that non-developing embryos might preferentially benefit to kin. In addition, *T. cristatus* larvae show no interest for dying embryos (Pierre Joly, personal communication), which are anyway still protected from consumption by egg capsules. The balanced lethal system of crested newts is thus very likely to be maladaptive.

From our analyses, such a system might have evolved during a sex chromosome turnover induced by environmental changes. This outcome actually occurred with high likelihood in some of our simulations sets, whenever the following two conditions were met. First, a polymorphism must pre-exist on the Y chromosome, with different haplotypes having fixed different deleterious mutations (such that homozygotes are lethal, while heterozygotes are viable and fully fertile). This corresponds quite precisely to the situation documented in *Poecilia reticulata* (Haskins et al. 1970 and references therein), where three Y haplotypes that segregate in natural populations, coding for different color morphs, have been shown to be homozygous lethal. Morphologically differentiated Y chromosomes have been documented in other fish species (e.g. Felip et al. 2004). Given the high drift and frequent selective sweeps expected to occur in Y chromosomes (owing to reduced effective sizes and absence of recombination), specific mechanisms might be required for the long-term maintenance of such polymorphisms. Sexual selection is a potential candidate: in natural populations of *Poecilia parae*, for instance, female preference for rare morphs mediates the coexistence of five distinct Y haplotypes, coding for distinct color morphs (Lindholm, Brooks, & Breden 2004; Hurtado-Gonzales & Uy 2010). Alternatively, such a polymorphism might stem from secondary contacts between isolated lineages.

Second, environmental changes with feminizing effects must eliminate the ancestral X chromosome before a new masculinizing mutation appears (so that the population passes through a transient state of TSD). In the case of *T. cristatus*, with known thermal dependence of sex ratios (Wallace & Wallace 2000), such a shift might simply arise from a temperature drop (stemming either from climatic change or from a range expansion). The condition for X elimination, however, was only met within a specific domain of population size and environmental variance (blue bars in Fig 2C). Too small population sizes and/or environmental variances induced extinctions during the TSD / biased sex-ratio episode (red bars in Fig 2C). By contrast, too large a variance (mostly at large N) prevented elimination of the X, which made populations turn back to the initial *XY* system after fixation of the masculinizing mutation *M*. For the same reason, the *XY* system was maintained throughout, whenever the masculinization mutation *M* appeared before *X* had any chance to be lost. These conditions, however, did not require particularly small population sizes (N from 500 to > 10'000), provided other conditions were met.

We assumed sex-reversed *XY* females to be fully fertile, which might not be the case of sex-reversed "*mM*" females (Wallace et al. 1997). Such an assumption, however, is conservative, because low-fertility *XY* females would actually increase the probability of loosing the X, and thereby the probability of fixation of the balanced lethal system. Once the X got lost and the population was in a Y_AY_B TSD system, it had to survive a period of female biased sex ratios, which, in some simulations, lasted for more than 2000 generations. This certainly reduced effective population sizes, but had little effect on population dynamics, given the promiscuous mating system of newts. Female biases in such cases might even boost population growth (Rankin & Kokko 2007), by increasing the absolute of reproducing females, for a fixed carrying capacity.

Hence, as our simulations shows, evolutionary outcomes as bizarre and seemingly maladaptive as the balanced lethal system of crested newts might actually be the inevitable consequence of a response to environmental changes, given some specific constraints on gene-environment interactions (namely, temperature dependence of sex-determination).

FIGURES

Figure 1: Quantitative-genetics model of sex determination with gene–environment interactions. The liability trait (sex factor) produced by genotype *IJ* increases with temperature *T*° (norms of reaction are assumed linear and parallel with slope β =1). Individual differences within populations (micro-environment differences during the sensitive period of embryonic development; Gaussian curve on the horizontal axis) translate into individual deviation from the genotypic mean (Gaussian curves on the vertical axis). Individuals develop into males if the sex factor exceeds a threshold (bold horizontal line), and into females otherwise. At initial conditions (*T*° = 0), genotypic values define a male-heterogametic system with *mm XX* females and *mm* $XY_{A,B.}$ males. Temperature decreases will lead to sex-ratio selection, favoring a masculinizing mutation (*M*). If *M* appears before the loss of X, it goes to fixation and the initial *XX XY* system is restored (genotypes in blue). If *M* appears after *X* is lost, a new male heterogametic system evolves on chromosome 4, with the fixation of a balanced lethal system on chromosome 1 (*mM* Y_AY_B, *mm* Y_AY_B).

Figure 1

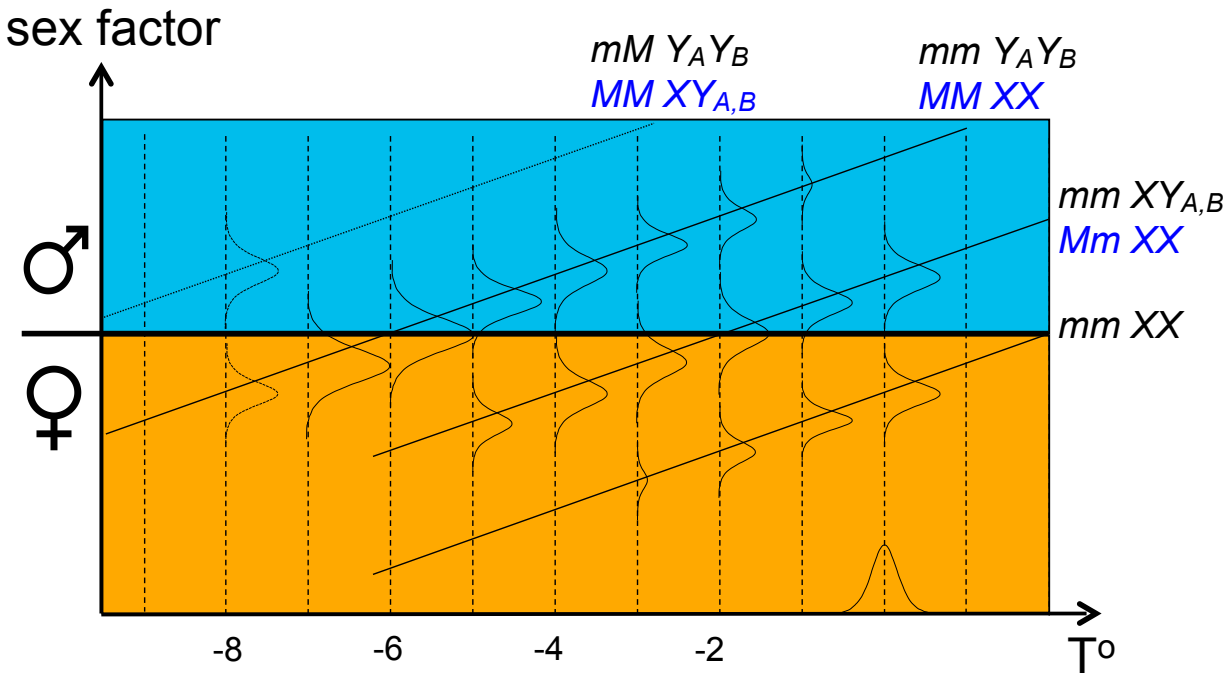
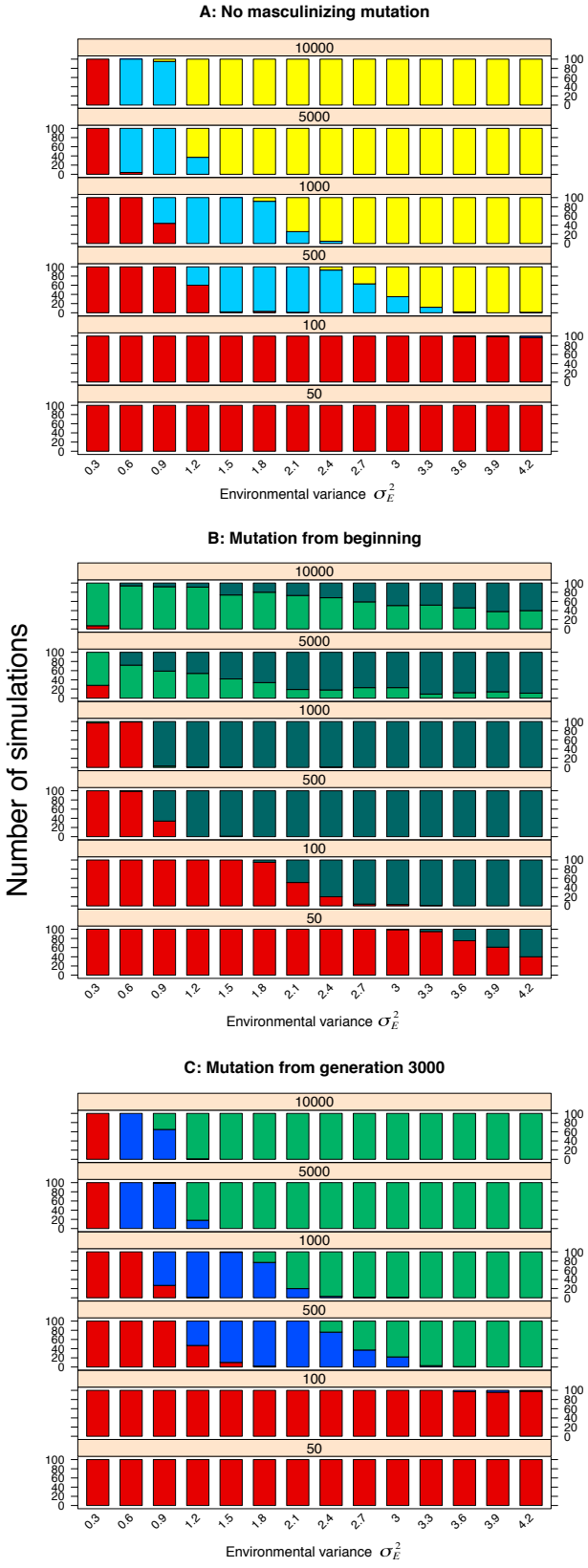


Figure 2. : Outcomes of simulations as a function of environmental variance, for six different population sizes (N= 50, 100, 500, 1'000, 5'000, 10'000) and three different mutation scenarios: (A) no masculinizing mutation, (B) masculinizing mutations possible from the start of the simulations, (C) masculinizing mutations possible after generation 3000. Color bars indicate the numbers of different outcomes out of 100 simulations. Red bars: extinction. Light blue bars: *mm* Y_AY_B fixed in both sexes (TSD with lethal system). Yellow bars: *mm* Y_AY_B males and females, *mm* $XY_{A,B}$ females (mixed female heterogamety with increased Y frequency and lethal system). Light green: *MM XX* females, *MM* $XY_{A,B}$ males (male heterogamety). Dark green: *MM XX* in both sexes (TSD without lethal system). Blue bars: *mm* Y_AY_B females, *mM* Y_AY_B males (male heterogamety on chromosome 4, with balanced lethal system on chromosome 1).



CHAPTER III

EVER YOUNG SEX CHROMOSOMES IN EUROPEAN TREE FROGS

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ABSTRACT

Non-recombining sex chromosomes are expected to undergo evolutionary decay, ending up genetically degenerated, as has happened in birds and mammals. Why are then sex chromosomes so often homomorphic in cold-blooded vertebrates? One possible explanation is a high rate of turnover events, replacing master sex-determining genes by new ones on other chromosomes. An alternative is that X-Y similarity is maintained by occasional recombination events, occurring in sex-reversed XY females. Based on mitochondrial and nuclear gene sequences, we estimated the divergence times between European tree frogs (*Hyla arborea*, *H. intermedia*, and *H. molleri*) to the upper Miocene, about 5.4–7.1 million years ago. Sibship analyses of microsatellite polymorphisms revealed that all three species have the same pair of sex chromosomes, with complete absence of X-Y recombination in males. Despite this, sequences of sex-linked loci show no divergence between the X and Y chromosomes. In the phylogeny, the X and Y alleles cluster according to species, not in groups of gametologs. We conclude that sex-chromosome homomorphy in these tree frogs does not result from a recent turnover but is maintained over evolutionary timescales by occasional X-Y recombination. Seemingly young sex chromosomes may thus carry old-established sex-determining genes, a result at odds with the view that sex chromosomes necessarily decay until they are replaced. This raises intriguing perspectives regarding the evolutionary dynamics of sexually antagonistic genes and the mechanisms that control X-Y recombination.

AUTHOR SUMMARY

Non-recombining sex chromosomes, such as the Y chromosome, are expected to degenerate over evolutionary times because they accumulate deleterious mutations that cannot be corrected by recombination with a pristine copy. In most cold-blooded vertebrates, such as frogs, however, sex chromosomes are undifferentiated. Why is that so? On the one hand, the “high-turnover” hypothesis holds that these sex chromosomes are regularly replaced before they had time to decay. On the other hand, the “fountain-of-youth” hypothesis posits that they are regularly rejuvenated by X-Y recombination in sex-reversed XY females. Here, we show that three species of tree frogs that diverged more than 5.4 million years ago share the same pair of undifferentiated sex chromosomes. Although male recombination stopped before species divergence, X and Y alleles show no differentiation, and cluster by species, not gametologs. We conclude that their sex chromosome homomorphy is not due to a recent turnover but is maintained over long evolutionary times by occasional recombination. Such rare episodes of X-Y recombination are expected to have long-lasting consequences on the evolution of sex chromosomes and sex antagonistic genes.

INTRODUCTION

The highly decayed Y chromosome of mammals results from an evolutionary process that started some 170 million years ago (mya), when a new masculinizing gene (*SRY*) first appeared on an autosome (Lahn & Page 1999; Graves 2006). Recombination then stopped in males in the vicinity of this new sex-determining gene, presumably to preserve epistatic interactions with sexually antagonistic mutations (Rice 1996). Genes that happened to be trapped in the non-recombining segment accumulated deleterious mutations under the combined forces of genetic drift, selective sweeps, background selection, and Muller's ratchet (Charlesworth & Charlesworth 2000). Similar processes are thought to have occurred in birds (Lawson-Handley et al. 2004), where females are the heterogametic sex, carrying a degenerated, non-recombining (W) chromosome. The seemingly ineluctable decay induced by the lack of recombination has led to the suggestion that sex chromosomes are "born to be destroyed" (Steinemann & Steinemann 2005), though a prevailing opinion is that gene loss slows down over time (Charlesworth & Charlesworth 2000) and that gene content might still show rapid evolution in old sex chromosomes (Hughes et al. 2010).

However, in sharp contrast with birds and mammals, decay and differentiation are rarely observed in cold-blooded vertebrates. Sex chromosomes have been described as homomorphic in about 96% of amphibians studied so far (Eggert 2004), and similar numbers are found in fishes (Devlin & Nagahama 2002). Even recognizing that seemingly homomorphic chromosomes might show some differentiation at finer scales, the contrast with warm-blooded vertebrates is striking. Why is that so? Two alternative models propose contrasting explanations. On the one hand, the "high-turnover" hypothesis suggests that master sex-determining genes are regularly replaced by new ones, so that the non-recombining segments that later evolve around the new sex-determining gene do not have enough time to degenerate (Volff et al. 2007). Direct evidence for recent turnover events is indeed accumulating (Tanaka et al. 2007; Cnaani et al. 2008; Ross et al. 2009), with different heterogametic systems found in closely related species, or even in populations from the same species (Miura 2008). However, it is not clear whether such events occur often enough to account for the overwhelming prevalence of sex-chromosome homomorphy. Phylogenetic analyses of amphibians have identified only seven heterogametic transitions during the evolutionary history of this species-rich group (Hillis & Green 1990), which certainly leaves enough time for the Y or W to diverge, even assuming that some turnovers did not affect heterogamety.

On the other hand, the "fountain-of-youth" hypothesis (Perrin 2009) holds that sex-chromosome integrity can be maintained over long evolutionary times by occasional recombination in XY females. Sex-

reversal experiments have shown that sex differences in the recombination patterns of several vertebrate and invertebrate species depend on phenotypic sex, not on genotype (Inoue, Fukumori, & Hiroyoshi 1983; Wallace et al. 1997; Lynn et al. 2005; Campos-Ramos, Harvey, & Penman 2009). The sex-reversed XY females of medaka fish display female-specific recombination patterns, while sex-reversed XX males show the characteristic male absence of recombination (Matsuda et al. 1999; Kondo et al. 2001). Similar patterns occur in frogs (Matsuba et al. 2010). As sex reversal occasionally occurs in ectotherms (due to the temperature dependence of physiological processes underlying sex determination, Dournon et al. 1990; Baroiller et al. 2009a; Grossen et al. 2011), the ensuing recombination in XY females should oppose Muller's ratchet and prevent the evolutionary decay of sex chromosomes.

Model System and Specific Predictions

Here we use European tree frogs to test contrasting predictions from these two models. All Eurasian tree frogs have homomorphic sex chromosomes (Anderson 1991). Male heterogamety was first evidenced in *Hyla arborea* by sex differences in the allelic distribution of microsatellite markers (Berset-Brandli et al. 2006; Berset-Braendli, Jaquiery, & Perrin 2007). Mapping linkage groups through sibship analyses identified nine sex-linked markers, which all revealed complete absence of male recombination, despite overlapping X-Y allelic distributions (Berset-Brändli et al. 2008). Similarity between gametologs was further confirmed by cDNA sequences of a sex-linked transcription cofactor: apart from some frame-preserving indels in polyglutamine repeat tracts (which are known for their high rate of slippage mutation), the X and Y copies showed no single base substitutions over 2,400 bp, including >800 synonymous sites (Niculita-Hirzel, Stöck, & Perrin 2008).

Is this striking X-Y similarity maintained by occasional recombination, or does it result from a recent turnover, followed by the rapid loss of male recombination? To test between these two alternatives, we combined investigations on gene genealogies and recombination patterns in two species from the sister clade to *H. arborea*, namely the Italian *H. intermedia* and the Iberian *H. molleri* (Stöck et al. 2008). The recent turnover model predicts that the sex chromosomes will differ between *H. arborea* and its sister-group species (as is observed, e.g., in medakas, sticklebacks, or tilapias, Tanaka et al. 2007; Cnaani et al. 2008; Ross et al. 2009). Markers shown to be sex-linked in *H. arborea* are thus expected to display both autosomal localization and normal male recombination in the sister species, while their genealogies (Figure 1b) should conform to the species genealogy (Figure 1a). If, however, the sex chromosomes are ancestral, these markers should display sex linkage and absence of male recombination in all three species (Figure 1c–e). Furthermore, under the X-Y-

recombination model, gene genealogies should conform to species genealogy (so that alleles cluster according to species; Figure 1c), while the opposite outcome (clustering by gametologs) would occur if X-Y recombination definitely stopped before species divergence (Figure 1d). Note that if a recent turnover occurred on ancestral sex chromosomes (with, e.g., the *H. arborea* proto-Y derived from the ancestral X, Graves & Peichel 2010), then markers in sister-group species should also be sex-linked but alleles should cluster by gametologs (Figure 1e).

RESULTS

Species Divergence Times

Phylogenetic analyses of mitochondrial and nuclear genes showed that *H. arborea* diverged from the sister species (*H. intermedia and H. molleri*) during the late Miocene, namely around the Messinian salinity crisis. Estimates point to lower Messinian (7.1 my, 95% HPDI 2.3 – 15.8 my) for the mtDNA cytochrome b (Figure 2) and upper Messinian (5.4 my, 95% HPDI 1.4 – 12.3 my) for intronic sequences of the nuclear *fibrinogen alpha* gene.

Sex-Specific Linkage Maps

Several of the nine microsatellites found to be sex-linked in *H. arborea* could be cross-amplified (six in *H. intermedia* and six in *H. molleri*). We genotyped a total of 111 families from the three species, each comprising a mating pair and an average of 20 offspring, plus a few additional non-mating adults (Table S1). Sibship analyses revealed shared synteny and complete linkage in males (Table S2a–d). In females, by contrast, pairwise recombination rates were very high (most of them between 0.30 and 0.50). These patterns did not differ between species (Morton M-test, Morton 1956), so that the three datasets could be pooled to produce a consensus map (Figure 3). Parsimony implies that male recombination stopped before the species diverged. This provides sufficient time to allow detectable sequence differentiation between non-recombining X and Y chromosomes, as otherwise found at nuclear and mitochondrial sequences (Figure 2).

Sex Linkage

The linkage groups in Figure 3 map to sex chromosomes in all three species. Despite the scarcity of sex-diagnostic alleles, sex linkage could be established on two grounds. First, significant sex differences in allelic frequencies were found at several loci in all species (Text S1). Second, sibship analyses and multilocus associations provided evidence for the coexistence of several different non-recombining Y haplotypes in natural

populations (Text S2 and Table S3). In all cases, autosomal localization (Figure 1b) could be rejected with high confidence.

Patterns of X-Y Similarities

Finally, we found higher X-Y similarity within the three species than between them, however we assessed it. First, size differences between conspecific X and Y alleles were smaller than between alleles randomly sampled at the same locus from different species (Figure S1), implying shorter coalescence times. Second, patterns of cross-amplifications depended on species more than on gametologs (Table S4), implying higher primer-sequence similarity between conspecific sex chromosomes than heterospecific gametologs. Third, the X and Y sequences of two sex-linked loci, chosen for their distant localization on the sex chromosomes (93.8 cM in the female consensus map) clustered according to species, not gametologs (Figure 4).

DISCUSSION

We conclude that all three species studied inherited the same pair of XY sex chromosomes from a common ancestor but that, despite absence of recombination in males, Y chromosomes show higher sequence similarities and overlap in allele frequency distributions with conspecific X chromosomes than with allospecific Y chromosomes. Hence, sex-chromosome homomorphy in *H. arborea* does not result from a recent turnover event, from either an autosome (Figure 1b) or an ancestral sex chromosome (Figure 1e). Local gene conversion between X and Y chromosomes (Figure 1e) occasionally occurs in mammals (Slattery, Sanner-Wachter, & O'Brien 2000) but cannot parsimoniously account for the large-scale X-Y similarity found in all markers and species, with respect not only to the sequence data (Figure 4) but also to the patterns of allelic sizes (Figure S1) and cross-amplifications (Table S4) at genotyping markers. Our data thus support occasional X-Y recombination (Figure 1c), occurring either in males or in sex-reversed XY females.

The maintenance of a potential for X-Y recombination over evolutionary times contrasts sharply with our failure to measure any recombination in males (Figure 3), raising important issues regarding the underlying mechanisms (Marais & Galtier 2003). Recombination in *H. arborea* males is suppressed on all sex-linked markers and drastically repressed on autosomes (Berset-Braendli et al. 2007), arguing against local mechanisms such as inversions (Andolfatto, Depaulis, & Navarro 2001). Genome-wide effects with phenotypic-sex dependence are likely to stem from meiotic or epigenetic processes (Tease & Hultén 2004). Meiosis in frogs occurs at very different times and under different physiological conditions in male and female germ cell lineages (Ogielska

2009), while imprinted genomic regions in humans are known to display large sex differences in recombination rates (Smalley 1993; Pàldi, Gyapay, & Jami 1995).

Our findings have important implications for the evolutionary dynamics of sex chromosomes. Given the high rate of female recombination documented here (Figure 3), a single event of sex reversal is expected to generate a wide diversity of new Y haplotypes. In the absence of male recombination, the fittest ones (i.e., those purged of the deleterious mutations that accumulate during periods of non-recombination, but still having the male-beneficial alleles at sexually antagonistic loci) should be sorted out by natural or sexual selection and spread among natural populations within a few generations. This interplay of recombination and selective sweeps might account for the significant differences in allelic frequencies, despite low sequence differentiation, between X and Y chromosomes. Phylogeographic studies of Y haplotypes over the range of *H. arborea*, which recently expanded into Western Europe from a West-Balkanic glacial refugium (Stöck et al. 2008), might help in uncovering historical signatures of such events. Signatures might also be found at the genomic level, with peaks of X-Y divergence in the vicinity of sex-determining or sex-antagonistic loci, which might be detected by looking at the coalescence times of neutral markers (Kirkpatrick, Guerrero, & Scarpino).

From our results, seemingly "young" sex chromosomes may harbor old sex-determining genes. The sex-determination system shared by these tree frog species may thus considerably predate their divergence. It will be interesting to study species further apart in the phylogeny (e.g., *H. savignyi, H. meridionalis*, or *H. japonica*, Stöck et al. 2008). In a wider perspective, similar investigations focusing on sister groups of species from other taxa, sharing the same pair of undifferentiated sex chromosomes, might allow estimates of the extent to which X-Y recombination contributes to the overwhelming prevalence of sex-chromosome homomorphy among cold-blooded vertebrates.

The fountain-of-youth and high-turnover hypotheses, however, are not to be seen as exclusive alternatives. The same mechanisms responsible for sex reversal and X-Y recombination (e.g., temperature shift stemming from a range expansion) may also generate turnover events via sex-ratio selection (Grossen et al. 2011), and the homomorphy maintained by occasional recombination may create favorable conditions for sex chromosome turnovers from other mechanisms, such as sex-antagonistic selection (van Doorn & Kirkpatrick 2007).

MATERIALS AND METHODS

Animal Sampling and DNA Extraction

The resource pedigree consisted of 2,863 individuals from 111 known family groups, each including a mother, father, and an average of 20 offspring per family (Table S1). Mating pairs caught in amplexus in the field were allowed to spawn; then buccal cells were sampled (Broquet et al. 2007) before release. A few additional crosses between *H. arborea* populations were produced in the lab (Table S1). Clutches (one per mating pair) were maintained in the laboratory until tadpoles had grown enough to allow tissue sampling (tip of tail). Buccal swabs and tissues were stored at −20°C before analysis. DNA was extracted using a QIAGEN DNeasy Tissue Kit following the manufacturer's protocol with few additional steps or using the BioSprint robotic workstation (QIAGEN). DNA was eluted in a 200 µl volume (QIAGEN Buffer AE) and stored at −18°C.

Microsatellite Primers, Amplifications, and Scoring

We used published primer sequences (Arens, Westende, & Bugter 2000; Berset-Brandli et al. 2006; Berset-Braendli et al. 2007; 2008; Berset-Brändli et al. 2008) except for Ha M2 and Ha M3, which, together with Ha 5–22, correspond to poly-Glutamine chains within different exons of the sex-linked gene HaMed15, and for which we designed primers based on the published X and Y sequences (GenBank EU276188 and EU276189) (Niculita-Hirzel et al. 2008). Ha M2 (F: 5′ GCC TGT TGA GCT GCT TGC 3′; R: 5′ GGG CAG TGC AAG CTC AGC 3′) ranges from 100 to 120 bp and has a complex motif including CAG, CAA, and GCA repeats. Ha M3 (F: 5′ CTG GTT TTG CTG TTG CTG AA 3′; R: 5′ TCA AGT CAC CCA GCA GAA TG 3′) has a size ranging from 175 to 185 bp and a complex motif including CAG and CAA repeats. Multiplex PCRs were carried out for the two loci in a total reaction volume of 10 µl containing 0.2 µM of each primer, 0.6× of Multiplex PCR Master Mix (QIAGEN), and 3 µl of extracted DNA. PCR amplifications were performed on the GeneAmp PCR Systems 2700 and 9700 (Perkin Elmer, Norwalk, CT) according to the following thermal conditions: initial denaturation at 95°C for 15 min followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 1 min 30 s, elongation at 72° for 1 min, and then a final elongation step at 60°C for 30 min. The same conditions were used to successfully amplify these two markers in *H. molleri* and *H. intermedia*.

For other primers, PCR reactions were conducted in two independent multiplex reactions (QIAGEN) co-amplifying up to six microsatellites (Berset-Braendli et al. 2008; Berset-Brändli et al. 2008), except for marker Ha 1–60 in *H. molle*ri and markers Ha 5–22, Ha H-108, and Ha D-110 in *H. intermedia*, which were amplified individually as follows: 10 µl reaction volume each containing 0.25 mM dNTP, 0.5 µM of each primer, 1× QIAGEN PCR Buffer (with MgCl2 15 mM), 0.2 mM MgCl2 (0.5 mM MgCl2 for Ha D-110 and no MgCl2 for Ha 5-22 and Ha

H-108), 1× QIAGEN Q-Solution, between 0.03 U and 0.1 U QIAGEN Taq, and between 1 and 3 µl of extracted DNA. PCR reactions were performed on GeneAmp PCR Systems 2700 and 9700 (Perkin Elmer, Norwalk, CT) according to the following thermal profiles: initial denaturation at 95°C for 15 min (94° for 5 min for individual amplification with QIAGEN Taq) followed by 32–35 cycles at 94°C for 45 s (QIAGEN Taq: 40–45 cycles), annealing at 58°C for 45 s (60°C for Ha H-108 in *H. intermedia* and Ha 1-60 in *H. molleri*), elongation at 72°C for 1 min, and a final elongation step at 60°C for 30 min (QIAGEN Taq: 75°C for 5 min). PCR products were analyzed on an automated sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystems). Allele sizes and genotypes were determined using GeneMapper 4.0 (Applied Biosystems) followed by manual proofreading. In order to confirm homology, alleles from each microsatellite locus were cloned and sequenced in all three species.

Population-Genetics and Linkage Analyses

Allele frequencies in males and females were calculated with FSTAT 2.9.3.2 (Goudet 1995). Linkage analyses were performed with CRIMAP 5.0 (Green, Falls, & Crooks) using the same procedures as in Berset-Brändli et al. (2008). Heterogeneity in recombination rates among populations and species was tested for each available marker interval with Morton's M-test (Morton 1956). In absence of heterogeneity, sample sets were pooled with the option *merge*.

Amplification, Cloning, and Alignment of Sequences

The mitochondrial cytochrome b gene was amplified with primers L0 and H1046 (Stöck et al. 2008). PCR products were sequenced in both directions, visualized on an ABI 3730 sequencer, and aligned with SEQUENCHER 4.9.

To amplify ca. 545 bp of intron 1 of *fibrinogen A*, *alpha-polypeptide*, we used two primers (MVZ47: 5′_AGTGAAAGATACAGTCACAGTGCTAGG_3′; MVZ48: 5′_GGAGGATATCAGCACAGTCTAAAAAG_3′) and a protocol developed by Jason B. Mackenzie in the Museum of Vertebrate Zoology (University of California, Berkeley). PCR were carried out in 12.5 µl reactions containing 7.55 µl H2O, 1.25 µl of PCR buffer including 1.5 mM MgCl2, 0.1 µl of dNTPs, 0.1 µl Taq QIAGEN, 0.75 µl of each primer having a concentration of 10 µM, and 2 µl of genomic DNA with a concentration of 20 ng/µl. For subsequent cloning, two of such reactions from each individual were pooled to increase volume. The PCR protocol followed a “touch-up” approach with 10 cycles of increasing annealing temperatures (55°C to 60°C) by 0.5 degrees each cycle (with 30 s at 95°C, 30 s at annealing temperature, and 45 s at 72°C), followed by 25 cycles with 30 s at 94°C, 30 s at 56°C, and 45 s at 72°C, and a final extension of 7 min at 72°C.

The sex-linked gene HaMed15 (ca. 1 kb fragments including 2 exons and 2 introns) was amplified with primers Ha 5-22F (5′-TTACAGCAACAGCAAATGG-3′) and p984R (5′_CGAGTATGCTTAATAGCTAATGCTA_3′). PCRs (94°C 1.5 min, 37×(94°C 45 s, 55°C 45 s, 72°C 1 min), 72°C 5 min) were carried out in 25 µl reaction volumes containing 17.75 µl H2O, 2.5 µl of PCR buffer including 1.5 mM MgCl2, 1.1 µl of a solution containing 2.0 mM MgCl2, 0.25 µl of dNTPs, 0.4 µl Taq QIAGEN, 0.5 µl of each primer having a concentration of 10 µM, and 2 µl of genomic DNA with a concentration of 10 ng/µl.

The sex-linked non-coding marker Ha A-103 was amplified (ca. 510 bp) with primers Ha A-103F1 (5′_GCCTAGAAATGTGCAGTGATC_3′) and Ha A-103R2 (5′_TGGAAAGTTTGCCCATTCAT_3′). PCRs (94°C 1.5 min, 40×(94°C 45 s, 50°C 54 s, 72°C 40 s), 72°C 5 min) were carried out in 25 µl reaction volumes containing 19 µl H2O, 2.5 µl of PCR buffer including 1.5 mM MgCl2, 0.25 µl of dNTPs, 0.25 µl Taq QIAGEN, 0.5 µl of each primer having a concentration of 10 µM, and 2 µ µl of genomic DNA with a concentration of 10 ng/µl.

For all nuclear markers, PCR products were cloned using the pGEM-easy vector system (Promega). Concentrations were first quantified (NanoDrop ND-1000 spectrometer) and adjusted to 25 ng/µl. We mixed 1.5 µl of template, 0.075 µl of vector (50 ng/µl), 2.5 µl 2× ligation buffer, 0.5 µl T4 ligase, and 0.425 µl water and ligated overnight (10°C). Transformations were carried out by incubating a mixture of 2.5 µl ligation mix and 12–25 µl JM109 High Efficiency competent cells for 20 min on ice and then heat-shocking them for 45 s at 42°C. Transformed cells were recovered in SOC medium for 1 h 30 min; 80–100 µl of cell suspension was applied to LB agar plates supplied with Ampicillin/IPTG/X-Gal. After incubation (18 h, 37°C), templates from a number of 10–12 white colonies were amplified with forward and reverse vector-specific primers M13. Nested vector-specific primers T7 and SP6 (Promega) were used as sequencing primers. All clones were sequenced in both directions and visualized on an ABI 3730 sequencer and aligned with SEQUENCHER 4.9. For all sex-linked markers we sequenced 10–12 clones from each individual to minimize the risk of allelic dropout; alleles were aligned and screened for singletons to correct for PCR error. Sequences included in phylogenetic analyses are thus represented by multiple clones each. GenBank accession numbers for HaMed15 sequences are JF317989 to JF318012; for the microsatellite-containing sequence Ha A103: JF318144 to JF318169; for intron 1 of the *fibrinogen A, alpha-polypeptide*: JF318013 to JF318047. Those for cytochrome b are provided in Table S5.

Phylogenetic Analyses

Maximum likelihood (ML) phylogenies were generated with PhyML 3.0 (Guindon & Gascuel 2003) using the GTR model for cytochrome b and HKY model for sex-linked (Ha A-103, HaMed15) and autosomal (Fibrinogen alpha) nuclear markers. For each case, we chose a BioNJ tree as a starting tree and used the combined subtree

pruning and regrafting (SPR) plus nearest neighbor interchange (NNI) options for tree improvement. All other parameters were set as default (http://atgc.lirmm.fr/phyml/). Bootstrap values were based on 1,000 resampled datasets.

Molecular Dating

Divergence times were estimated assuming an uncorrelated exponential relaxed molecular clock. For the mitochondrial cytochrome b gene, we assumed a normal distribution of priors for the substitution rate, with mean 0.01 my−1 (±0.007 SD) (Mulcahy & Mendelson 2000; Rowe, Harris, & Beebee 2006), and a GTR plus gamma model of sequence evolution (Modeltestserver 1.0). We used a Yule tree prior (constant speciation rate per lineage) as most appropriate for species-level divergences (Drummond et al.). DNA sequence data were analyzed both with and without codon partition, with different partitions for codons 1+2 and 3 (results turned out to be very robust regarding partitioning). For the *fibrinogen alpha* gene (intron 1) we followed the same approach but used a HKY plus Gamma model of sequence evolution (Modeltestserver 1.0) and normal prior distributions for substitution rates with mean values ranging 0.001 to 0.002 my−1 (Hoegg et al. 2004). Analyses were run for 20 Mio generations each and repeated to ensure stability of estimates.

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FIGURES

Figure 1. Expected gene genealogies under different evolutionary scenarios. The focal gene is localized either on an autosome (green) or on a sex chromosome (red) in *H. arborea* (*Ha*), *H. intermedia* (*Hi*), or *H. molleri* (*Hm*). Arrows indicate turnovers in sex-determination systems. (a) Reference genealogy for an autosomal or mitochondrial marker. (b) In *H. arborea*, the marker lies on a proto sex chromosome recently derived from an autosome. Sex linkage is restricted to *H. arborea*, and genealogy conforms to species genealogy. (c) The marker is on ancestral sex chromosomes and thus sex-linked in all three species, but its genealogy still conforms to species genealogy due to occasional X-Y recombination. (d) The marker is on ancestral sex chromosomes and thus sex-linked in all three species, but due to absence of X-Y recombination, alleles cluster according to gametologs, not species. Within gametologs, gene genealogy conforms to species genealogy. (e) In *H. arborea*, the marker lies on a proto sex chromosome recently derived from an ancestral sex chromosome (dashed arrow), such that HaY clusters with the ancestral HaX. The marker is sex linked in all three species, but in the sister group of *H. arborea*, alleles cluster according to gametolog, not species. Note that a similar genealogy would result from local gene conversion (see Figure 1 in Pecon et al. 2000).

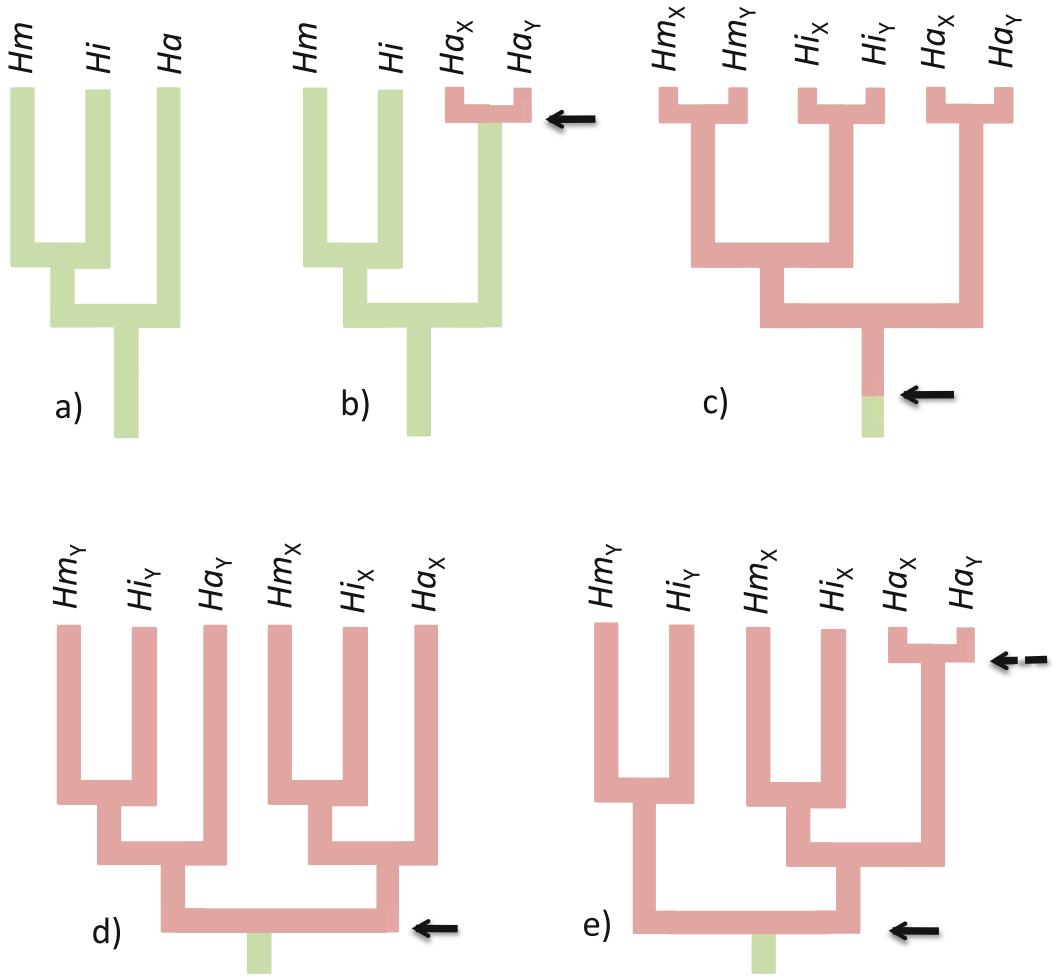


Figure 2. Maximum-likelihood phylogeny for tree frog cytochrome b lineages. The divergence time between mtDNA cytochrome b lineages of *H. arborea* and sister-group species (complete sequences of ca 1000 bp, multiple samples across species geographic ranges) averages 7.1 my (2.3–15.8 my 95% HPDI). Origin of samples and GenBank accession numbers are provided in Table S5.

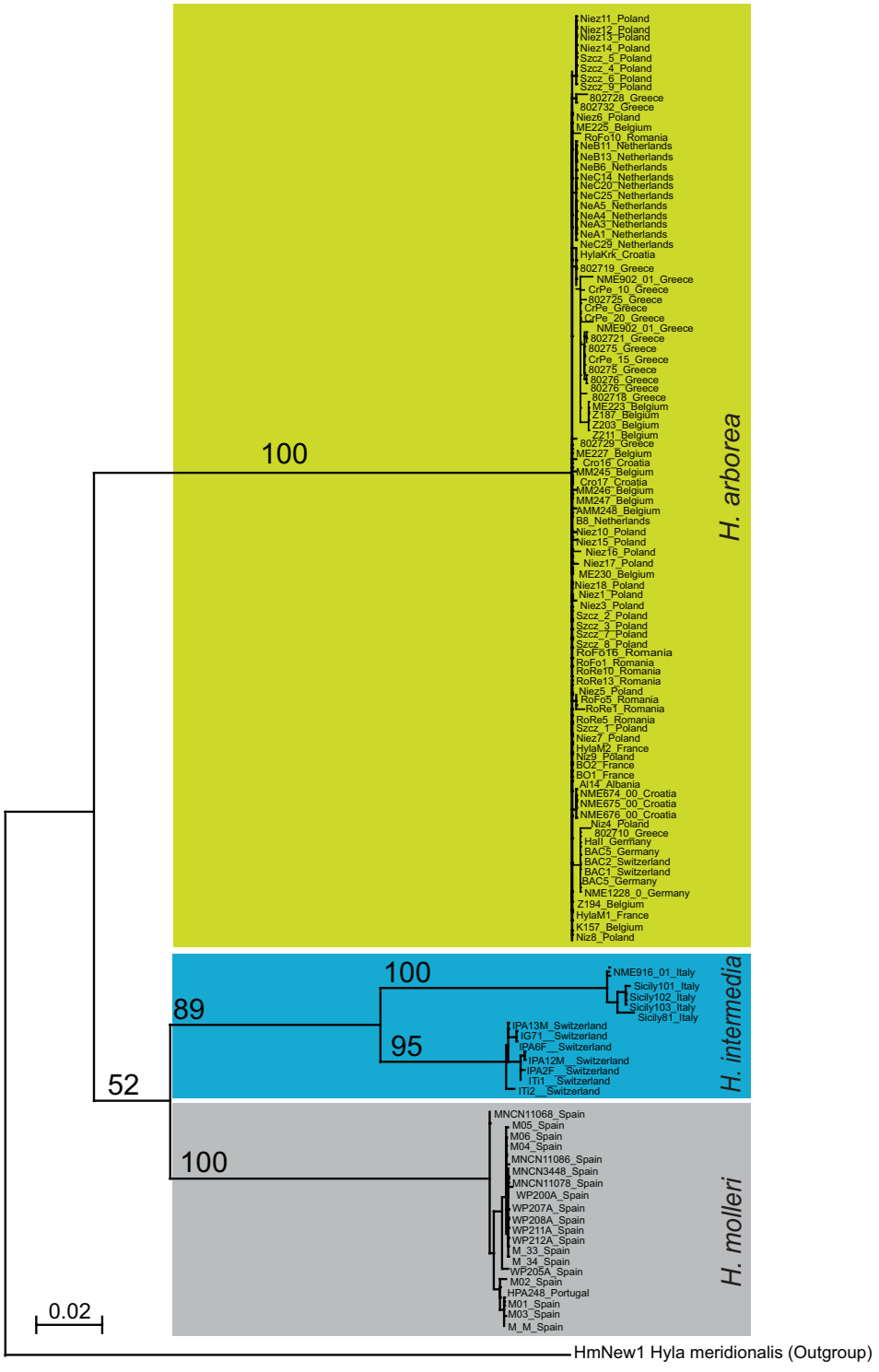


Figure 3. Recombination maps for sex-linked markers. The complete absence of recombination in males (right) contrasts sharply with the high recombination rates found in females (left). Lengths are given in cM units for the consensus map, and correspondences are provided graphically for species-specific maps. In each case the map is the one with highest likelihood, except that for *H. intermedia*, ranking third but with a log-likelihood very close to (and not significantly lower than) the first one (−120.79 versus −119.71).

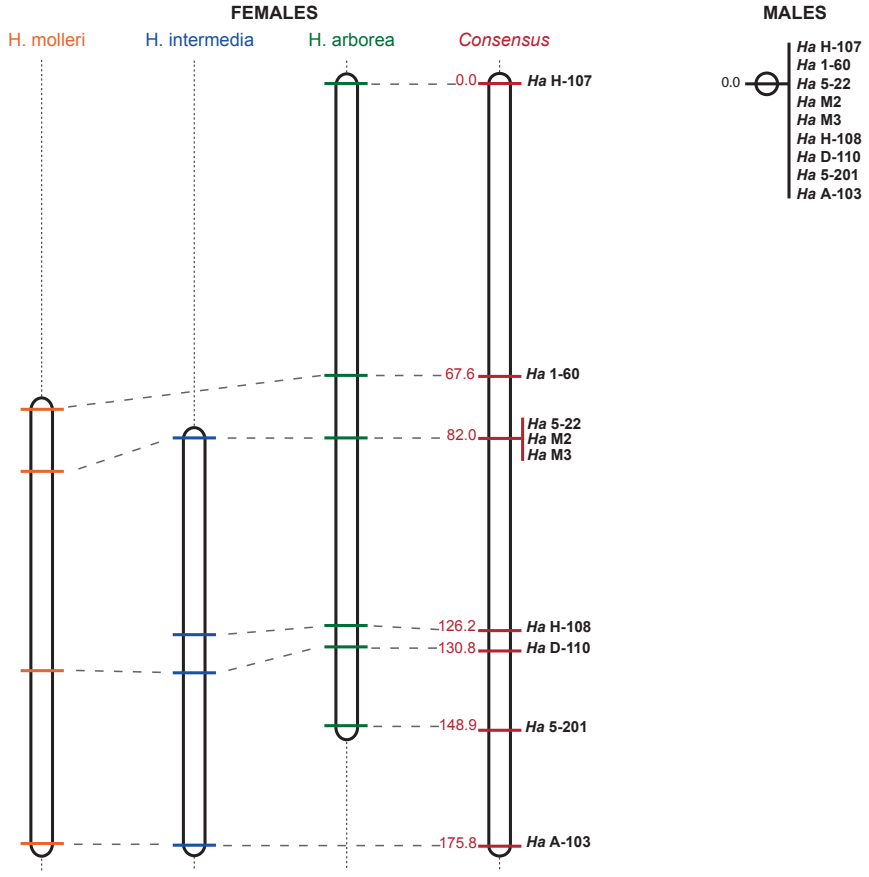
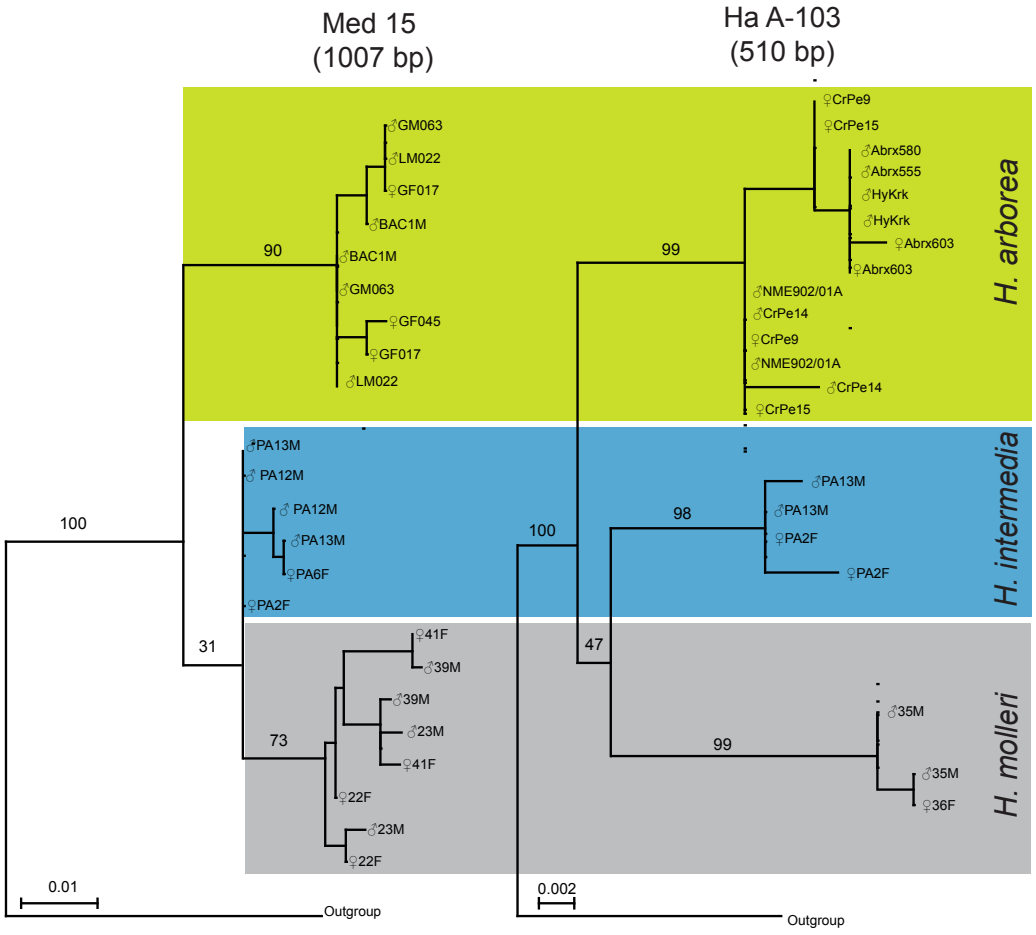


Figure 4. Gene genealogies for two sex-linked loci. The transcription cofactor HaMed15 (left, ca. 1 kb sequences with two introns and two exons, including the marker Ha 5–22) and the non-coding Ha A-103 (right, ca. 510 bp sequences) are 93.8 cM apart on the female recombination map (Figure 3). For both markers, the X and Y alleles (marked with the same label when amplified from the same male) cluster by species, not by gametolog. Bootstrap values are higher for the non-coding Ha A-103 (≥98%) than for the highly conserved transcription cofactor HaMed15 (≤90%), and higher for *H. arborea* than for species from its sister group.



SUPPLEMENTARY INFORMATION

Available online:

http://www.plosbiology.org/article/info%3Adoi%2F10.1371%2Fjournal.pbio.1001062#s5

CHAPTER IV

EVOLUTIONARILY STABLE RATE OF SEX REVERSAL

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ABSTRACT

Contrasting with birds and mammals (which display purely genetic sex determination and highly decayed sex chromosomes), sex determination in cold-blooded vertebrates is characterized by homomorphic sex chromosomes and frequent dependence on temperature. According to the "fountain of youth" model, these two features are linked: Occasional temperature-dependent sex reversal allows X-Y recombination in sex-reversed XY females, thereby purging the Y chromosome from its accumulating load of deleterious mutations. We formalize sex determination systems in a quantitative genetics framework to investigate evolutionary forces on sex reversal, assuming a male heterogametic system in which X-Y recombination stopped in males to benefit from epistatic interactions with sex antagonistic genes. As our simulations show, X and Y chromosomes show divergent evolutionary interests. The accumulation of deleterious mutations on the Y chromosome selects for a decrease in the masculinizing effect of the male-determining allele (to favor XY sex reversal), but a concomitant decrease in the feminizing effect of the female allele on the X (to restrict XY sex reversal). The evolutionarily stable rate decreases with the strength of sex-antagonistic selection, and is overall very low (one sex-reversed XY female per population every few generations). This, however, is far enough to prevent the decay of the Y chromosome.

INTRODUCTION

Mammals and birds display purely genotypic sex determination and highly differentiated sex chromosomes. In both groups, a decayed chromosome (Y and W respectively) characterizes the so-called heterogametic sex (males and females respectively). In contrast, many reptiles, including turtles and crocodiles, have no such chromosomes, sex being determined via the external temperature experienced by the developing embryo.

Temperature-dependent sex determination (TSD) has long been seen as fundamentally different from genotypic sex determination (GSD), relying on specific and exclusive mechanisms (Valenzuela et al. 2003). However, the alternative view is now emerging that these two modes of sex determination actually constitute the two ends of a continuum (Sarre et al. 2004). This view has gained impetus from recent advances in molecular biology, showing that the pathways of sex-determination are fundamentally conserved across both vertebrates (Graves & Peichel 2010) and invertebrates (Kato et al. 2011), and from the discovery of an increasing number of species in which both genes and temperature interact to determine sex (Conover & Heins 1987; Quinn et al. 2007; Radder et al. 2008; Pen et al. 2010).

Quantitative genetics offers a unifying framework to formalize such a continuum (Bulmer & Bull 1982; Grossen et al. 2011). Sex qualifies as a threshold trait, underlain by a continuous liability factor (e.g. a sex hormone), such that individuals develop into males if the liability factor exceeds a fixed threshold, and into females otherwise. This liability trait is under both genetic and environmental control. More specifically, genotypes define norms of reaction (Fig. 1a-c), corresponding to the amount of the liability factor produced as a function of environment (temperature). Depending on environment, a given genotype may develop into either male or female. The phenotypic variance of the liability trait has thus both a genetic component (stemming from the coexistence of different genotypes within populations) and an environmental component, arising from the different microenvironments experienced by individuals from the same population.

At one extreme, the system tends to GSD (Fig. 1a) if two genotypes (e.g. XY and XX), coding for liability trait values far apart from the threshold within the normal temperature range, segregate in a population. Phenotypic sex correlates then perfectly with genotype (i.e., the variance in phenotypic sex is only genetic). At the other extreme, the system tends to TSD (Fig. 1c) if one single genotype is fixed in the population, producing either sex depending on individual microenvironments. Here the variance in phenotypic sex is purely environmental. In-between are mixed systems (Fig. 1b), in which part of the phenotypic variance is due to genes,

and part to environment. Phenotypic sex correlates partly with genotype, some individuals being "sex reversed"; i.e., they develop into one sex, despite having a genotype that most often produces the other sex (e.g., XY females).

GSD, TSD and mixed systems are widespread among vertebrates, and their phylogenetic distribution among reptiles and fishes suggests that transitions occur on a regular basis (Devlin & Nagahama 2002; Mank & Avise 2009; Pokorna & Kratochvil 2009; Gamble 2010). This raises the question of what ultimate causes drive, on an evolutionary timescale, a given lineage into one or the other solution: What are the fitness costs and benefits of different sex-determination systems?

On the one hand, GSD offers a straightforward way to produce even sex ratios, which are evolutionary stable in large, free-mixing populations. It also offers an efficient way to solve sex-antagonistic conflicts, and thereby facilitates the evolution of sexual dimorphism (Rice 1984). Consider a phenotypic trait taking values that are beneficial to one sex, but detrimental to the other. If both the sex and the trait are coded by genes that co-segregate, then the male-beneficial allele will be statistically associated to maleness, and the female-beneficial allele to femaleness. As a result, sex-antagonistic genes are often located on sex chromosomes, in close association with sex-determination genes. Coloration genes, for instance, are tightly linked to the sex-determination locus in sexually dimorphic fishes from a diversity of families (Aida 1921; Kallman 1970; Houde 1992; Tripathi et al. 2009; Roberts, Ser, & Kocher 2009). Bright coloration is favored in males by sexual selection, and dull coloration in females by natural selection (e.g. Endler 1980; 1983; Brooks & Endler 2001; Lindholm & Breden 2002; Kingston, Rosenthal, & Ryan 2003).

For the same reason (namely, to fully benefit from epistatic interactions), sex-antagonistic genes select for an arrest of recombination in the heterogametic sex (as recombination stops, the male-beneficial allele always occurs in males, and only in males). However, this also comes with a cost: deleterious mutations accumulate on the non-recombining Y or W chromosomes, under the combined evolutionary forces of genetic drift, background selection, selective sweeps and Muller's ratchet (Charlesworth & Charlesworth 2000). GSD systems that have remained stable over long evolutionary times (e.g. 170 my in mammals) display this characteristic decay of sex chromosomes, with evolutionary strata testifying to a progressive expansion of the non-recombining segment (Lahn & Page 1999; Lawson-Handley et al. 2004). The human Y chromosome now contains less than one hundred genes, mostly involved in testis development and male fertility. These remnant genes are regularly affected by recurrent deleterious mutations, despite mechanisms specifically evolved to counteract their decay (namely, YY and XY gene conversion; e.g. Kuroda-Kawaguchi et al. 2001; Trombetta et al.

2010; Marais, Campos, & Gordo 2010). This Y chromosome has already disappeared from some mammalian lineages (Just et al. 1995; Sutou, Mitsui, & Tsuchiya 2001; Arakawa et al. 2002;).

TSD, on the other hand, provides no automatic mechanism to produce even sex ratios. Specific additional processes are required, relying e.g. on female choice of oviposition sites (Doody et al. 2006; Doody 2009;). Given such behavioral fine-tuning, TSD might theoretically outcompete GSD when optimal sex ratios differ from even (as occurs under local mate competition). Similarly, TSD might be favored whenever temperature affects phenotypes and fitness in a sex-specific way (Charnov & Bull 1977; Conover 1984; Warner & Shine 2008; Pen et al. 2010). Besides this specific situation, however, TSD offers little opportunity to build sexual dimorphisms, and no way to solve sex conflicts through genetic dimorphisms. But for the same reason, sex-antagonistic genes cannot induce an arrest of recombination in the heterogametic sex, so that TSD species do not suffer from the associated gene decay.

On one hand, therefore, the direct selective pressures stemming from sex-antagonistic genes are expected to favor GSD systems, with contrasting genotypic values, far apart from the threshold (plain arrows in Fig. 1b). This should minimize the rate of sex reversal, i.e. the probability that sex antagonistic alleles end up in individuals of the wrong sex. On the other hand, as further argued below, the arrest of recombination and accumulation of deleterious mutations induced by sex-antagonistic genes should counteract this trend, and favor increased rates of sex reversal (doted arrows in Fig. 1b).

An important point to make in this respect is that recombination rates (both on sex chromosomes and on autosomes) depend on phenotypic sex, not on genotype. In TSD species (such as the saltwater crocodile *Crocodylus porosus*; Isberg et al 2006), recombination patterns differ between sexes, despite absence of genetic differentiation. In GSD species, sex reversals occurring either naturally (Matsuba et al. 2010) or experimentally (e.g. Inoue et al. 1983; Wallace et al. 1997; Matsuda et al. 1999; Kondo et al. 2001; Campos-Ramos et al. 2009) consistently show that the phenotypic sex (as opposed to genotypic sex) is crucial in determining the patterns of recombination on both autosomes and sex chromosomes. GSD systems that have remained stable over long evolutionary times (e.g. mammals) have accumulated structural differences between the X and Y chromosomes that prevent their recombination in sex-reversed XY females, but autosomal recombination still show this phenotypic-sex dependence (Lynn et al. 2005). Hence, X-Y differentiation in such cases is presumably the consequence of a long absence of recombination, not its cause, which is more likely to be found in meiotic or epigenetic processes (Perrin 2009). It follows that X-Y recombination, occurring in sex-reversed females, is

expected to purge deleterious mutations and prevent the decay of Y chromosomes (the "fountain of youth" model; Perrin 2009; Stöck, Horn, et al. 2011b).

These opposing selective forces, stemming from sex-antagonistic genes on the one hand, and from the accumulating deleterious load on the other hand, are expected to favor intermediate solutions (Fig. 1b). A moderate differentiation between norms of reactions should allow benefitting from epistatic interactions with sex-antagonistic genes, while still allowing rare events of sex reversal to regularly purge the accumulating deleterious load. More specifically, we expect the XX and XY genotypes to differ in their optimal rates of sex reversal, because incentives for recombination and purge are much stronger on the Y than on the X. In this paper, we use individual-based simulations to test these predictions, and ask whether and how rates of sex reversal might be optimized by natural selection.

MATERIALS AND METHODS

Model

Individual-based simulations were run with a modified version of the program quantiNemo 1.0.3 (Neuenschwander et al. 2008), using a simple life cycle with non-overlapping generations. At reproduction, offspring were produced in numbers matching the carrying capacity. For each offspring, a mother and a father were chosen randomly with replacement, corresponding to a promiscuous mating system as in (Grossen et al. 2011).

Each individual had a pair of sex chromosomes, comprising one sex-determining gene (SD), one sex-antagonistic gene (SA), and one hundred additional genes, potentially mutating to deleterious forms. The liability trait (A) was computed as the sum of the two allelic values at the sex-determining locus (i.e., genetic effects were additive) plus an environmental effect, randomly sampled from a normal distribution with average 0 and variance σ_E^2. The sex-antagonistic trait (B) was similarly affected additively by alleles at the sex-antagonistic locus. It influenced fitness in a sex-specific way according to the sigmoid functions (Fig. S1):

$$w_{B,m} = M + \frac{1-M}{1+e^{c(B_m - B)}} \quad (1a)$$

$$w_{B,f} = 1 - \frac{1-M}{1+e^{c(B_f - B)}} \quad (1b)$$

where M is the lower asymptote (minimum fitness value), B_f and B_m the inflection point of the curve (arbitrarily set to +0.5 and -0.5 for females and males respectively), and c the slope at this point (arbitrarily set to -1 and 1). Hence male fitness increased from M to unity with increasing B values, while female fitness decreased from unity to M (Fig. S1).

Fitness was also affected by deleterious mutations hitting the functional genes spread on the sex chromosomes. Offspring viability (v) was computed as the product over loci $v = \prod\prod v_i$, where $v_i = 1$, $1-s$ or $1-hs$ depending on whether the locus i was homozygous wild-type, homozygous mutant, or heterozygous, respectively. Following (Higgins & Lynch 2001), we defined dominance h as a negative function of the selection coefficient s: $h(s) = \frac{1}{(2+20s)}$, which implies that mutations with large effects are much more recessive than mutations with small effects. For a subset of parameter values, we also run simulations with h fixed to either 0.05 or 1, independent of s.

Recombination only occurred in females (independent of genotype). Functional genes were regularly distributed on the sex chromosome, 1 cM from each other (hence, total map length was 100 cM in females, versus 0 cM in males). The sex-determining locus was arbitrarily localized at one extremity of the chromosome, and the sex-antagonistic locus 30 cM away (Fig. 2).

Simulations

A first set of simulations was aimed at investigating the effect of sex reversal on the accumulation of deleterious mutations, both with and without sex-antagonistic selection. Alleles at the sex determining locus were fixed to X=-2 and Y=6, producing genotypic values -4 and +4 for XX and XY individuals respectively. Different rates of sex reversal were generated by varying the environmental variance (σ_E^2) from 0 to 2 (steps 0.2). Other parameter values were set to M=1 or 0.5 (sex-antagonistic selection), N=100, 1'000 or 10'000 (population size), and s=0.025 or 0.0125 (strength of selection against deleterious mutations). Deleterious mutations occurred at a rate $\mu=10^{-4}$ (reverse mutations to wild-type alleles were neglected). At the beginning of the simulations, every individual was mutation free. The sex-antagonistic gene was allowed to evolve, with mutations ($\mu=10^{-4}$) among a set of 13 alleles ranging -6 to +6, and a truncated uniform distribution of mutational steps.

We, furthermore, investigated in more detail pure GSD systems (with complete absence of sex reversal; environmental variance fixed to σ_E^2 =0). Sex-antagonistic selection was varied by changing the lower fitness

asymptote from M = 0 to 0.75 (step 0.25) and the strength of deleterious mutations from s = 0 to 0.8 (logarithmic scale: $s=0.8/2^n$, where $n=\{0,1,2...12,\infty\}$).

The second set of simulations was aimed at investigating evolutionarily stable rates of sex reversal under the opposing forces of sex-antagonistic selection and deleterious mutations. The sex-determining locus was allowed to mutate ($\mu=10^{-4}$) among 161 alleles ranging -8 to +8 (step 0.1) with a truncated normal distribution of mutational steps (variance =1.8). Environmental variance was fixed to $\sigma_E^2=1$. Sex-antagonistic selective pressure was varied from M = 0 to 0.75 (step 0.25) and the strength of deleterious mutations from s = 0 to 0.8 (logarithmic scale: $s=0.8/2^n$, where $n=\{0,1,2...12,\infty\}$). Population size was set to N=100, 1'000, or 10'000. Simulations were run for all possible combinations of parameters (fully factorial design).

RESULTS

Fixed SD alleles

In the first set of simulations, the proportion of loci fixing deleterious mutations progressively increased with time (Fig. 3), up to an equilibrium value set by the rate and strength of deleterious mutations, chromosomal effective population sizes (N_X or N_Y) and recombination rates (determined by environmental variance). Recombination was negligible for $\sigma_E^2 \leq 0.4$, so that the dynamics of deleterious mutations on the X and the Y were effectively decoupled. As a result, deleterious mutations accumulated on the Y (Fig. 4A), strongly affecting male fitness (Fig. 4B). For N=1'000, s= 0.0125 and $\sigma_E^2=0$, for instance, about 80% of loci on the Y fixed deleterious mutations (as opposed to 0% on the X; Fig. 4A), lowering mean male fitness to 0.63 (as compared to 0.98 in females; Fig. 4B).

For $\sigma_E^2 > 0.4$, some sex reversal and recombination occurred, at rates increasing with environmental variance. The mutation load on the Y was thereby much purged, reaching values similar to the X at $\sigma_E^2 = 2$ (Fig. 4A). As a result, male fitness was also much improved (Fig. 4B). One XY female every few generations was sufficient to purge most of the mutation load on the Y. For N= 1'000, M=1 and s=0.0125, for instance, one sex-reversal event every five generations (σ_E^2 = 1.4) lowered the proportion of deleterious mutations fixed on Y down to 1-2% (Figs. 3 and 4A).

In the absence of sex-antagonistic selection (*M* fixed to 1), allelic values at the sex-antagonistic locus evolved neutrally around an average of zero, with a large variance. With *M* fixed to 0.5, by contrast, these alleles evolved towards highly differentiated values (+6 and – 2 for the Y-linked and X-linked alleles, respectively; data not shown). This affected the purging process by depressing fitness in sex-reversed XY females (due to the male allele at the sex-antagonistic locus), thereby lowering the opportunity for X-Y recombination. Purging was thus less efficient on the Y (Fig. 3, white boxes; Fig. 4A, right panels), resulting in lower male fitness (Fig. 4B, right panels).

The more detailed simulations of a pure GSD system (σ_E^2=0) allowed better characterizing the crucial role played by different strengths (*s*) of deleterious mutations (Fig. 5), together with effective population size (*N*). Selection is expected to counteract drift for *sN* values exceeding one (Kimura 1983). Accordingly, increasing *s* induced a shift from a regime of accumulation to one of purge (Fig. 5A). Adult fitness thus first decreased with *s* (due to the increasing deleterious effect of accumulated mutations), then re-increased when reaching the domain of purge (Fig. 5B). The shift from accumulation to purge occurred at much higher *s* values for the Y than for the X (e.g., if N=1'000, *s*=0.025 for Y versus *s*=0.003125 for X), due to its lower effective size and absence of recombination. This induced discrepancies between male and female fitness curves at intermediate *s* values (Fig. 5B), providing incentives for the Y to recombine with the X.

Evolving SD alleles

In the second set of simulations, alleles at the SD locus were allowed to evolve. Equilibrium values for X and Y alleles are provided in Fig. 6A as a function of the selection coefficient *s*, for different intensities of sex-antagonistic selection (*M* varied from 0 to 0.75, step 0.25) and population sizes *N* (*N*= 1'000 or 10'000; values for *N*= 100 are provided in Figure S2).

In absence of incentives to recombine, sex-antagonistic genes prevented sex reversal: When deleterious mutations had similar dynamics on the X and on the Y (i.e., either accumulation at small *s*, or purge at high *s*; Fig. 5A), the Y allele at the SD locus evolved close to the maximal possible value (in average 7.0 and 7.5 for N=1'000 and 10'000, respectively), with the X allele co-evolving to values ensuring equal sex ratios (in average -2.5 and -2.8 respectively; Fig. 6A). For intermediate *s* values, by contrast, the faster accumulation of deleterious mutations on the Y induced a counter-selection for SD alleles closer to the threshold, allowing sex reversal to occasionally occur (Fig. S3).). Outcomes were qualitatively similar when *h* was fixed to either 0.05 or 1, independent of *s* (performed for N=1'000 and 10'000, *M*=0.75; data not shown).

This effect was very slight when strongly counteracted by sex-antagonistic selection (Fig. 6A, M= 0), but became more pronounced as sex-antagonistic selection weakened (e.g., M=0.75). The effects on male and female fitness are best seen by comparing figures 5B and 6B: While female fitness was only marginally affected, male fitness was largely improved by occasional sex-reversal events, mostly at low sex-antagonistic selection (M=0.75).

Interestingly, both Y and X alleles converged towards the threshold (Fig. 6A), but the latters to a lower extent. As a result, sex-reversed XY females were twice as more frequent as sex-reversed XX males (Fig. S3). These rates also depended on population size: At equilibrium (t = 100'000 generations), XX sex-reversal occurred at rates 0.03, $2.8*10^{-4}$ and $5.5*10^{-5}$ for N=100, 1'000 and 10'000 respectively, as compared to values of 0.06, $4.8*10^{-4}$ and $1.1*10^{-4}$ for XY sex reversals (average over M and s values). These rates also decreased as sex-antagonistic selection increased (from M=0.75 to M=0). Medians were still lower, due to the asymmetric distribution of equilibrium rates.

DISCUSSION

In addition to occasional environmental dependence, sex determination in cold-blooded vertebrates is characterized by the overwhelming prevalence of homomorphic sex chromosomes. All amphibian species studied so far display GSD, but 96% have homomorphic sex chromosomes (Eggert 2004). Similar numbers occur in fishes (Devlin & Nagahama 2002). Two non-exclusive mechanisms have been proposed to account for this widespread homomorphy, namely i) a high rate of sex-chromosome turnovers (Volff et al. 2007; Grossen et al. 2011; Stöck, Croll, et al. 2011a) and ii) occasional XY recombination (Perrin 2009).

The latter mechanism (the "fountain-of-youth") recently received empirical support from a study on tree frogs (Stöck, Horn, et al. 2011b). Several European tree frog species (*Hyla arborea*, *H. intermedia* and *H. molleri*) were shown to inherit the same pair of homomorphic sex chromosome from a common ancestor but, although recombination stopped in males before species divergence (i.e., > 5.4 mya,), X and Y show no sequence differentiation. The Y alleles of sex-linked loci are more similar to conspecific X alleles than to allospecific Y alleles, hence providing evidence for occasional XY recombination (Stöck, Horn, et al. 2011b). Naturally occurring sex reversal has already been documented in the closely related *H. japonica*: Beside XY females, (Kawamura & Nishioka 1977) report the capture of several YY males (necessarily born to fertile XY females).

Interestingly, these YY males were albinos, suggesting that the Y in this case had fixed a recessive mutation of a sex-linked coloration gene.

As our results show, such deleterious mutations should accumulate on non-recombining Y chromosomes at a pace depending on the rate (μ), strength (s) and dominance (h) of deleterious mutations, as well as on population size (N). The mutation rate implemented in our simulations (10^{-4}) seem reasonable: Empirical estimates range from 0.1 to 1 mutation per individual per generation for a diversity of taxa, from *Drosophila* and *Daphnia* to humans (reviewed in Lynch et al. 1999).

Regarding the strength of selection (s), the large set of values tested (from 0 to 0.8) covered the full range of dynamics, from accumulation (at small s) to purge (at high s). We focused our simulations on slightly deleterious mutations ($s < 0.05$) which have been shown empirically to represent the most frequent case (reviewed in Lynch et al. 1999). Though the value of s was fixed for any given set of simulation, similar studies have shown the dynamics of purge and accumulation to be very robust regarding this assumption, with qualitatively similar outcomes when s was drawn from an exponential, log-normal or Gamma distribution, rather than being kept constant (Jaquiery, Guillaume, & Perrin 2009; Gordo & Campos 2008).

Our assumption of a negative relationship between h and s is commonly made in similar modeling studies (Higgins & Lynch 2001; Jaquiery et al. 2009) and relies on large sets of empirical data (Deng & Lynch 1996; Phadnis & Fry 2005;). Further simulations with h fixed to 0.05 or 1 respectively, gave qualitatively robust outcomes.

Finally, population size (N) mattered mostly in interaction with s, because drift strongly opposes selection at low N values (e.g., Kimura 1983). As a result, the shift from accumulation to purge occurred at lower s values in larger populations (Fig. 5A, N=1'000 vs 10'000). This shift also occurred at lower s values for the X than for the Y chromosome (Fig. 5A), due to the threefold difference in effective size. However, the absence of recombination (Muller's ratchet) also contributed to this contrast, which would have been even stronger if we had implemented a polygynous mating system (which further contributes to lower the Y effective size) or a sex-specific mutation rate (which is often higher in males; reviewed in Hedrick 2007).

Such a contrast is in line with empirical observations from pure GSD systems (such as mammals and birds), where deleterious mutations accumulate on the non-recombining sex chromosomes, but are purged from the recombining one (reviewed in Graves 2006). This convergence with empirical patterns confirms that our simulations settings and parameters values were realistic.

Depending on parameter values, this accumulating mutation load had strong negative effects on male fitness, to the extent that small populations (N=100) went extinct at intermediate s values (Figs S2 to S4). This negative effect on male fitness induced strong incentives for the Y to purge its load via recombination with the X. This selected for less masculinizing Y values at the SD locus (i.e., closer to the threshold; Fig. 6A). Interestingly, the X alleles at this locus simultaneously converged towards the threshold (Fig. 6A), as a way to *prevent* sex-reversal and recombination. Indeed, there is a clear conflict of interest between the Y and the X, the latter spending two thirds of its life in females where it anyway recombines. The X chromosome has little benefits to recombine with the Y, because this increases its load of deleterious mutations (in addition to disrupting epistatic interactions with sex-antagonistic alleles). Hence, this upward shift in X values is to be interpreted as a way to counteract the downward shift in the Y alleles, in order to lower the probability that XY individuals develop into females.

Too strong an upward shift, however, would also be costly, because the fitness of sex-reversed XX males is lowered by their female-beneficial allele at the sex-antagonistic locus. Sex-antagonistic selection is indeed bound to play an important role in this context, by lowering the fitness of sex-reversed individuals (as documented e.g. in *Triturus cristatus*; Wallace et al. 1999). Weaker antagonisms (M=0.75 versus M=0) thus allowed more sex reversal, and thereby better purge and higher male fitness (Figs 6A, 6B and S3).

As a net result of these differential and counteracting forces, sex-reversed XY females were twice as more frequent as sex-reversed XX males (Fig. S3). As this figure also shows, however, the overall rates of sex reversal at equilibrium were very low. For N = 1'000, for instance, we expect about one XY female every 6-8 generations (median), calculated for the s value (s=0.0125) and sex-antagonistic selection (M=0.75) at which sex reversal was maximal. Still lower rates are actually enough to purge large parts of the load, and presumably keep the sex chromosomes homomorphic. This rarity may explain why sex reversal is only rarely documented in the field (e.g. Crew 1921; Witschi 1929a; Aida 1936; Kawamura & Nishioka 1977; Nagler et al. 2001; Matsuba et al. 2008). In the lab, it is easily triggered (as reviewed in Wallace et al. 1999; Eggert 2004; Ospina-Álvarez & Piferrer 2008), but usually occurs at extreme temperatures relative to specific natural ranges. In Tilapias (*Oreochromis niloticus*), for instance, feminization occurs below 20° C, and masculinization above 32° C (Bezault et al 2007; Baroiller et al 2009). In the newt *Triturus cristatus*, feminization occurs below 16°C, and masculinization above 24°C (Wallace and Wallace 2000). This should ensure that sex reversal only occurs at rare occasions.

Hence, as our present results suggest, sex reversal might not be the simple side effect of a physiological dysfunction, but be optimized by natural selection, as a way to purge deleterious mutations on the Y. Assuming

recombination to occur in females but not in males (as observed in *Hyla*; Berset-Brändli et al. 2008; Stöck, Horn, et al. 2011b), we expect an intermediate strategy to evolve under the opposing forces of sex-antagonistic selection and deleterious load.

An alternative solution to solve this conflict might consist in the maintenance of some male recombination. This process involves different proximate mechanisms (in particular regarding the control over recombination), and certainly has different evolutionary consequences. In the case of *Hyla*, for instance, a single event of sex reversal is expected to generate a wide diversity of new Y haplotypes, given the high rate of female recombination. In the absence of male recombination, the fittest Y haplotypes (i.e., those purged of the deleterious mutations that accumulate during periods of non-recombination, but still having the male-beneficial alleles at sexually antagonistic loci) should be quickly sorted out by natural or sexual selection, and spread among natural populations within a few generations. The evolutionary consequences of this interplay between sporadic recombination and selective sweeps should be quite distinct from those expected from a consistently low male recombination. It would be interesting to compare the two options through further simulation studies, in which male recombination rate is allowed to evolve.

Similarly, additional simulations would be required to investigate the conditions that allow either pure GSD (Fig. 1a) or pure TSD (fig 1c) to evolve. Some pure GSD lineages such as mammals have evolved YY and XY gene conversion from palindromic sequences (e.g. Rozen et al. 2003; Skaletsky et al. 2003; Rosser & Balaresque 2009; Hughes et al. 2010; Trombetta et al. 2010), which counteracts the accumulation of deleterious mutations (Connallon & Clark 2010; Marais et al. 2010), as well as dosage compensation to prevent the detrimental effects arising from gene inactivation on the Y chromosome (Charlesworth 1996; Payer & Lee 2008).

TSD species, on the other hand, have evolved alternative sets of strategies, including behavioral adaptations to control sex ratios (such as female choice of nest site; Doody et al. 2006), which may confer significant benefits over GSD when optimal sex ratios differ from even. Evolutionary benefits may also accrue to TSD whenever temperature affects phenotypes and fitness in a sex-specific way (Charnov & Bull 1977; Conover 1984; Warner & Shine 2008; Pen et al. 2010; reviewed in Janzen & Phillips 2006). Temperature-dependent sex-determination offers no way to build sex-antagonistic genetic polymorphism, but sexual conflict might be solved through differential gene expression (reviewed in Ellegren & Parsch 2007), although such complex adaptations are certainly slower to evolve than sex linked polymorphism and suppressed recombination in GSD (Scotti & Delph 2006).

Additional simulations, along the lines of the present study, but accounting for several of the assumptions that may or may not favor TSD versus GSD, would help shedding further light on the evolution of alternative sex-determination strategies.

FIGURES

Figure 1. Sex determination in a quantitative-genetics framework. The amount of sex factor (vertical axis) depends both on genotype (*XX* or *XY*, reaction norms increasing with temperature T°, horizontal axis) and on environmental variance (normal distribution around genotypic mean). a) GSD: genotypic means are far enough, each side from the threshold (horizontal bar) that sex is not affected by environmental variance (no sex reversal). b) Mixed system: genotypic means are close enough to the threshold that some sex reversal occurs (XX males or XY females; c) TSD: a single homozygous genotype is fixed in the population, so that sex is determined by environment only. Arrows in b) indicate selective pressure for reaction norms to diverge (plain arrows, due to sex-antagonistic genes) or to converge (dashed arrows, due to the accumulating load of deleterious mutations).

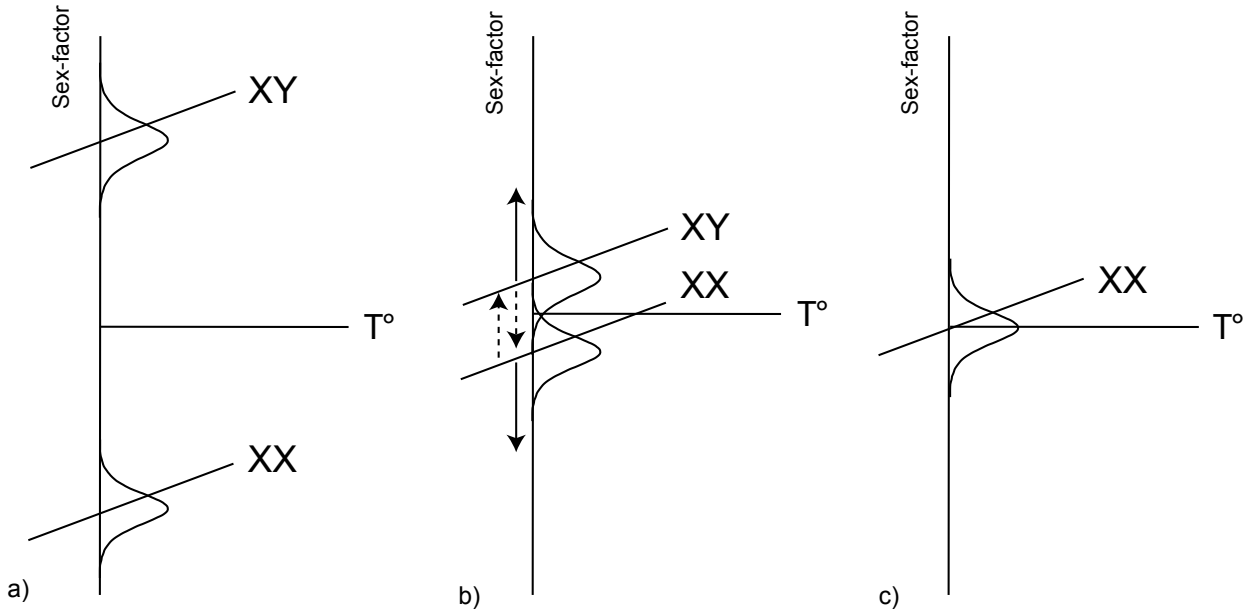


Figure 2. Male and female linkage map of the model sex chromosomes, in cM units. No recombination occurs in males (map length = 0 cM). In phenotypic females, the sex-determining gene (SD) was arbitrarily positioned at the end of the chromosomes (0 cM) and the sex antagonistic gene 30 cM away from it. One hundred potentially deleterious genes were evenly distributed every cM.

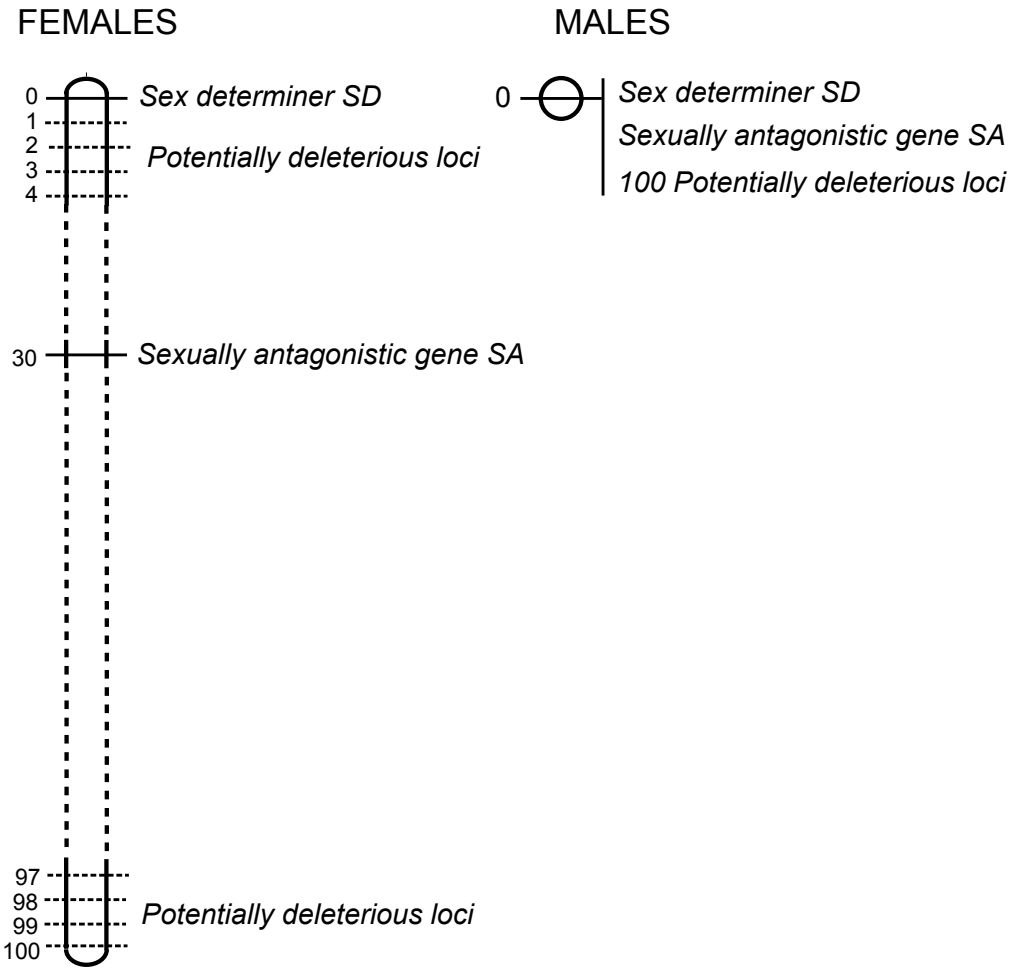


Figure 3. Proportion of loci on the Y that fixed deleterious mutations (vertical axis) as a function of time (horizontal axis). Mutations accumulate in absence of recombination (small σ_E^2 values), but are purged when sex reversal allows recombination with the X (large σ_E^2 values). Equilibrium values also depend on the presence (white boxes, M=0.5) or absence (grey boxes, M=1) of sex antagonistic selection. Other parameter values: N=1000, s=0.0125 and μ=10^{-4}. Box plots with median are delimited by 25th and 75th percentiles, and whiskers represent the 5th and 95th percentiles.

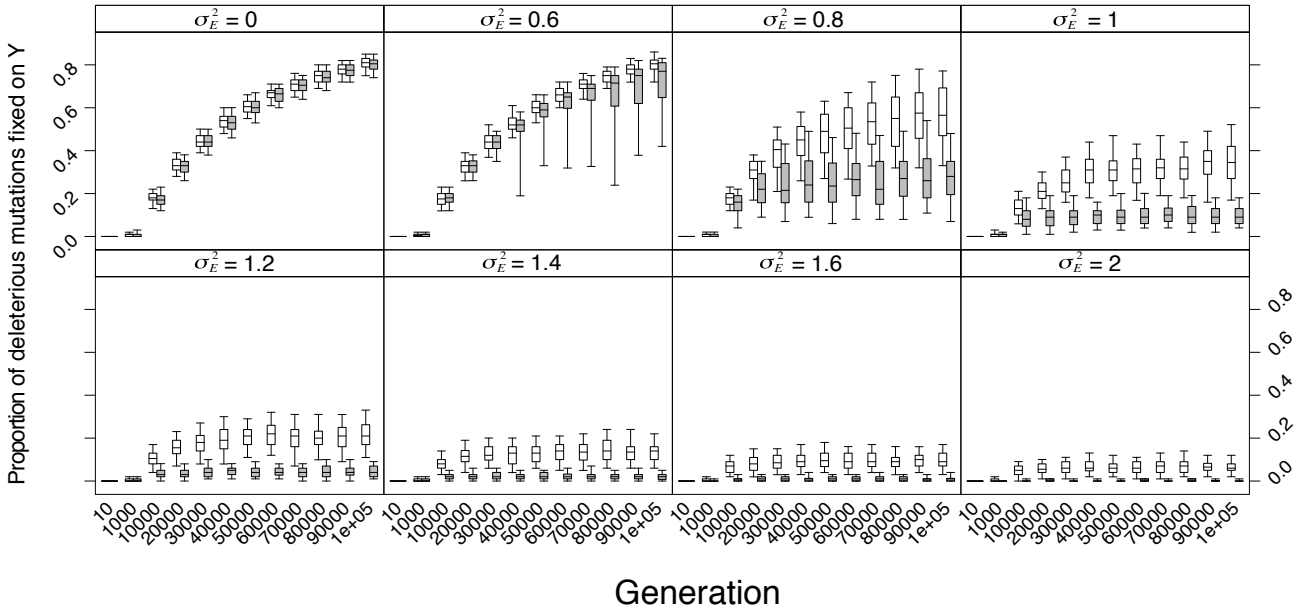


Figure 4. A) Equilibrium proportions of deleterious mutations fixed on X (white boxes) and on Y (grey boxes) and B) equilibrium fitness values for males (grey) and females (white), as a function of environmental variance σ_E^2 (horizontal axis). Mutations accumulate on the Y and depress male fitness when σ_E^2 is too small to allow sex reversal and recombination. Quantitative values also depend on whether sex antagonistic selection is present (right panels, M=0.5) or absent (left panels, M=1), as well as on population size (N=1'000 or 10'000). Other parameter values s=0.0125 and μ=10^{-4}. Box plots as in Figure 3.

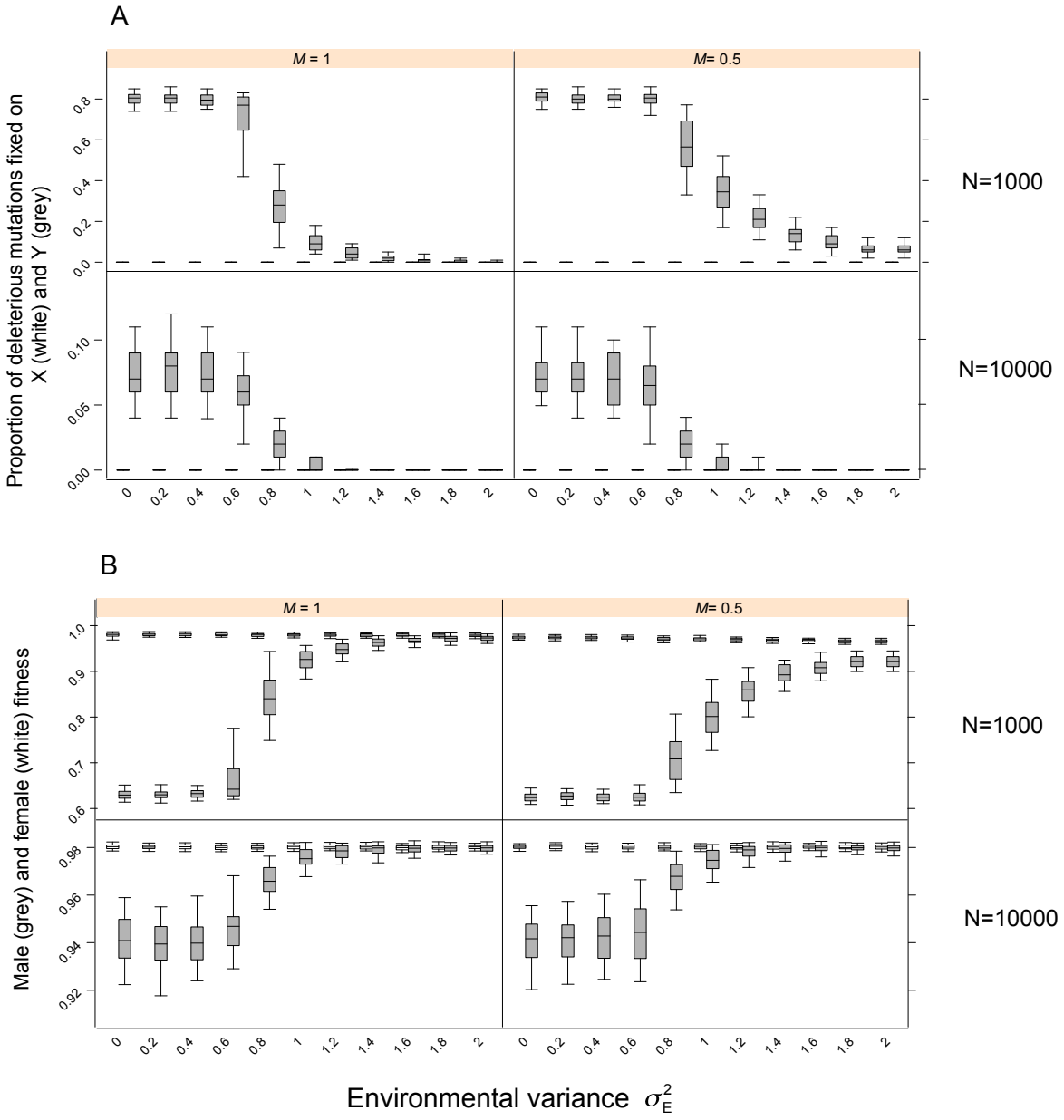


Figure 5. A) Equilibrium proportions of deleterious mutations fixed on X (white boxes) and Y (grey boxes) and B) equilibrium fitness values for females (white boxes) and males (grey boxes) for varying strength of deleterious mutation selection (s = 0 to 0.8, logarithmic scale on horizontal axis, with $s=0.8/2^n$, where $n=\{0,1,2...12,\infty\}$. The shift from accumulation to purge occurs at lower s values for the X than for the Y, and also for large than for small populations (N =10'000 vs 1'000), but sex-antagonistic selection had no effect (M = 0 to 0.75). Other parameter values σ_E^2 =0 and μ=10^{-4}. Box plots as in Figure 3. See Fig. S4 for a color version that also provides results for N=100.

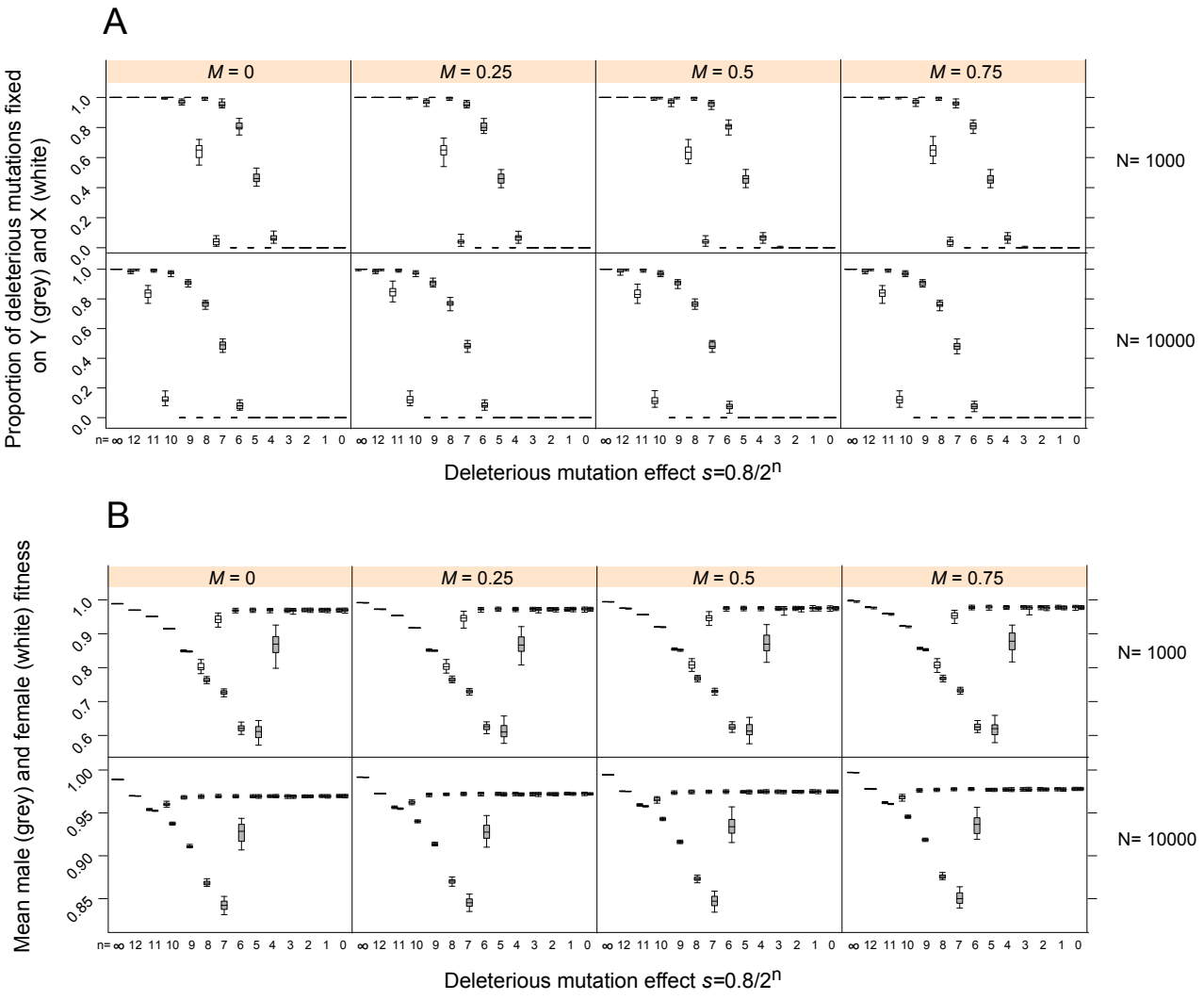
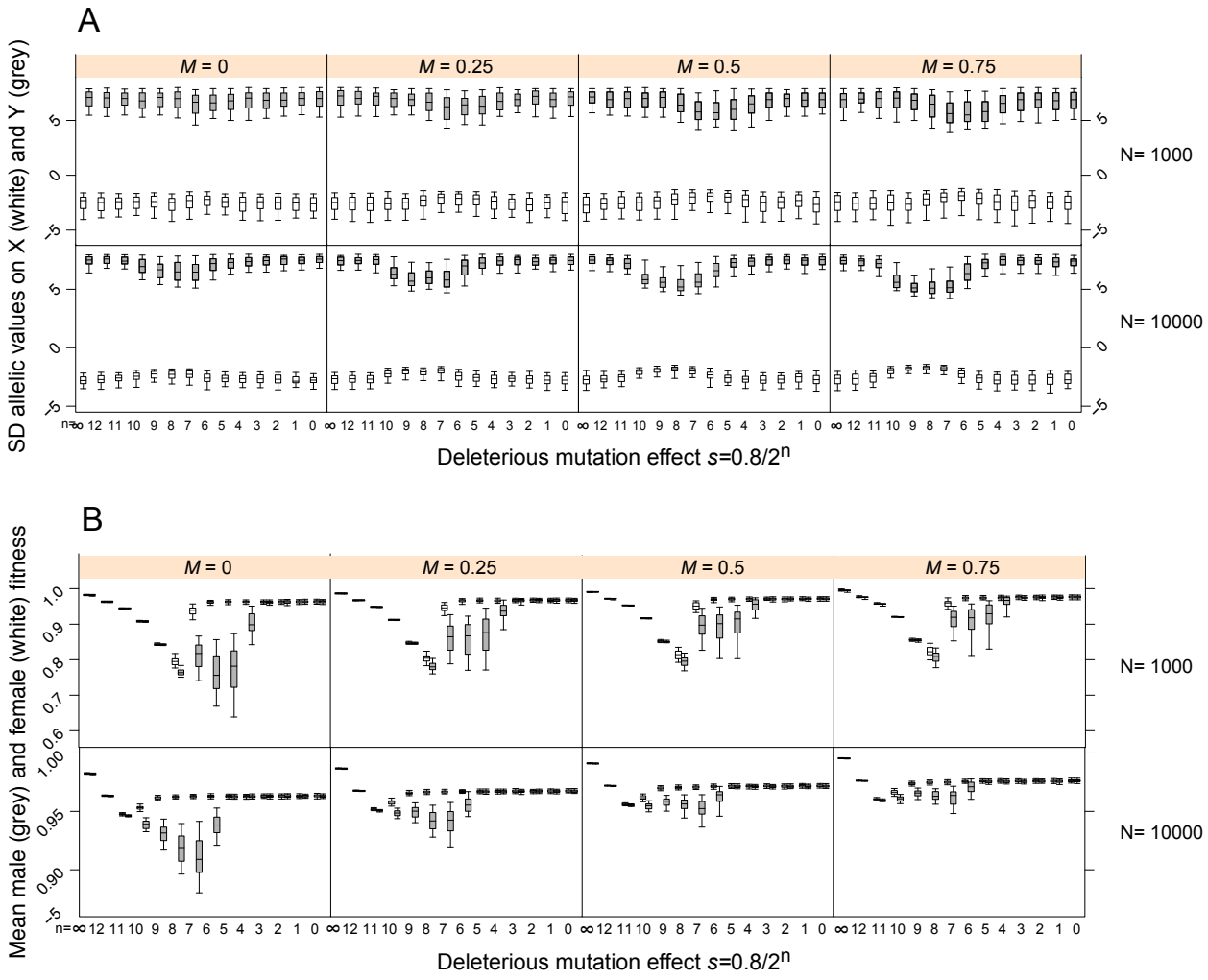


Figure 6. A) Equilibrium values for the X alleles (white boxes) and Y alleles (grey boxes) at the sex-determining locus, and B) equilibrium fitness for females (white boxes) and males (grey boxes) for varying strength of deleterious mutation selection (s = 0 to 0.8, logarithmic scale on horizontal axis with $s=0.8/2^n$, where $n = \{0,1,2...12,\infty\}$. The X and Y alleles converge towards the threshold at intermediate s values, which induces sex reversal and recombination, improving male fitness (compare with Fig.5). This trend is stronger in larger populations (N=10'000 vs 1'000) and at weaker sex-antagonistic selection (M=0.75 versus 0). Box plots as in Figure 3. See Fig. S2 for a color version that also provides results for N=100.



SUPPLEMENTARY MATERIAL

Figure S1. Sex-specific fitness under sex-antagonistic selection. Male fitness (dashed blue line) increases with trait value B (horizontal axis) from its minimal value (here M = 0.5) to its maximal value (1) with an inflection point at B_m = -0.5. Female fitness (plain red line) drops from 1 to M=0.5, with an inflection point at B_f = +0.5.

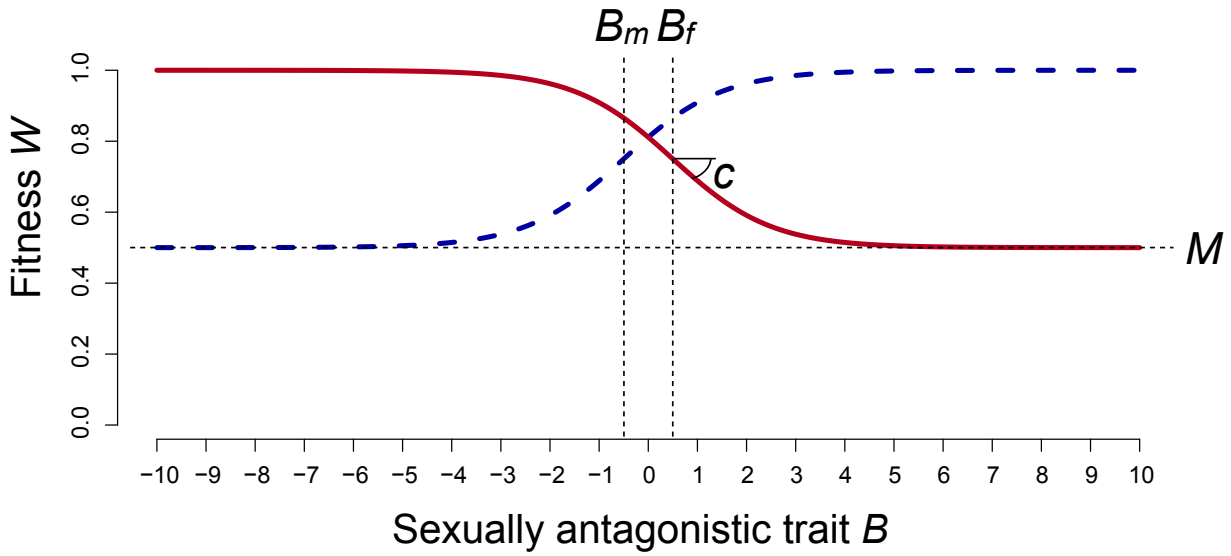


Figure S2. A) Equilibrium values for the X alleles (orange boxes) and Y alleles (green boxes) at the sex-determining locus, and B) equilibrium fitness for females (red boxes) and males (blue boxes) for varying strength of deleterious mutation selection (s = 0 to 0.8, logarithmic scale on horizontal axis with $s=0.8/2^n$, where $n=\{0,1,2...12,\infty\}$. The X and Y alleles converge towards the threshold at intermediate s values, which induces sex reversal and recombination, improving male fitness (compare with Fig.5). This trend is stronger in larger populations (N=10'000 vs 100) and at weaker sex-antagonistic selection (M=0.75 versus 0). Missing values at intermediate s values for N=100 are due to extinctions. Box plots as in Figure 3.

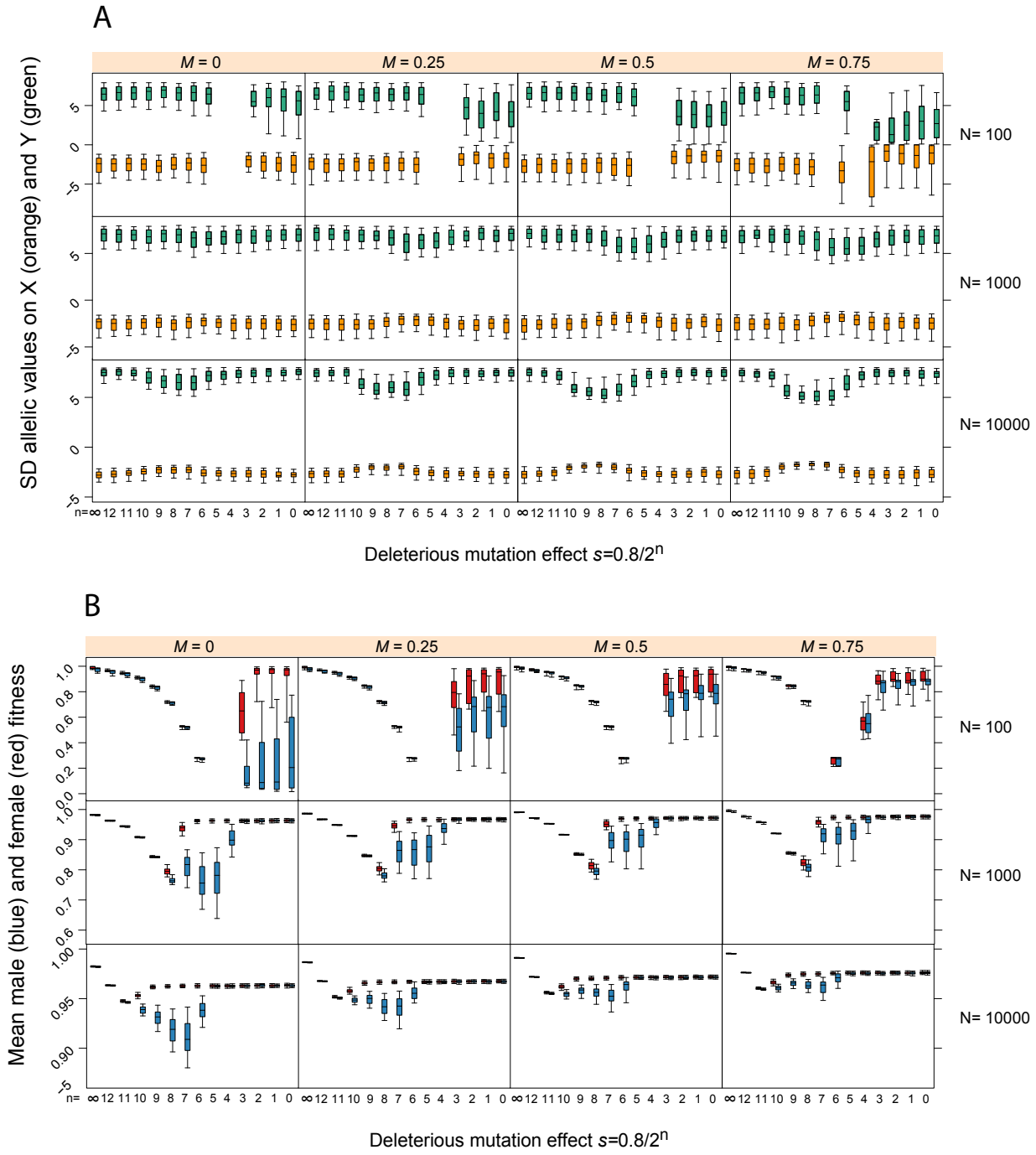


Figure S3. Equilibrium rates of sex reversed XX males (blue) and XY females (red) for varying strength of deleterious mutation selection (s = 0 to 0.8, logarithmic scale on horizontal axis with $s=0.8/2^n$, where n = (0, 1,.. 12 and ∞). Sex reversal occurred at intermediate s values, at rates decreasing with increasing sex-antagonistic selection (M from 0.75 to 0, right to left) and with increasing population size (N form 100 to 10'000, from top to bottom). Box plots as in Figure 3.

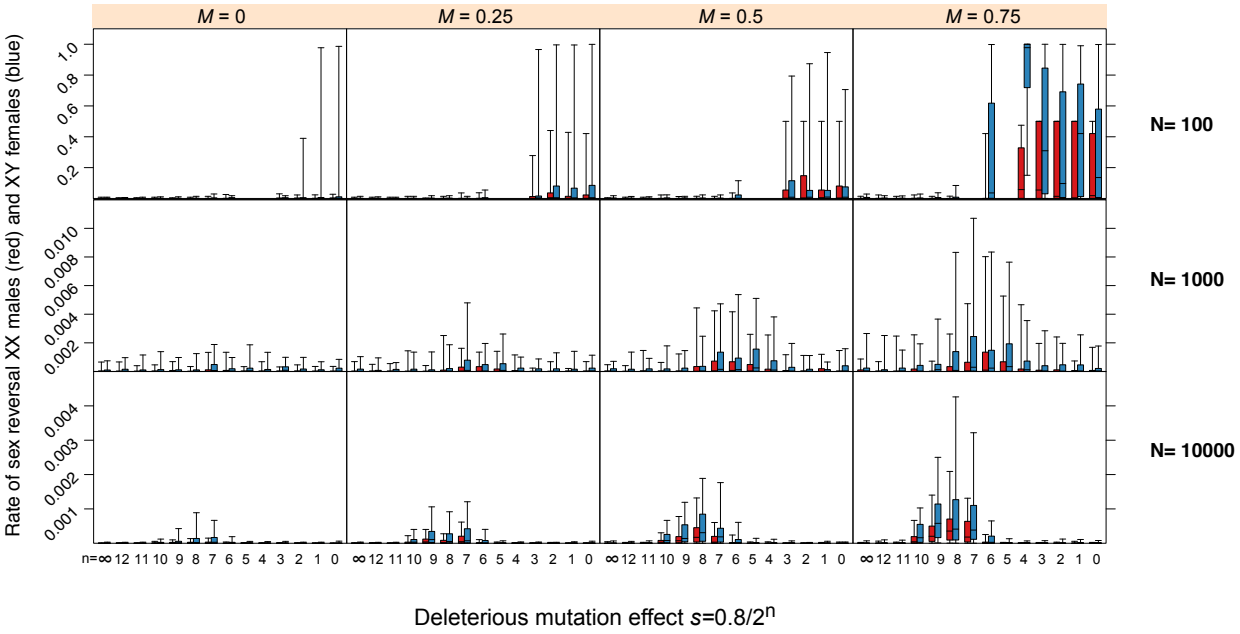
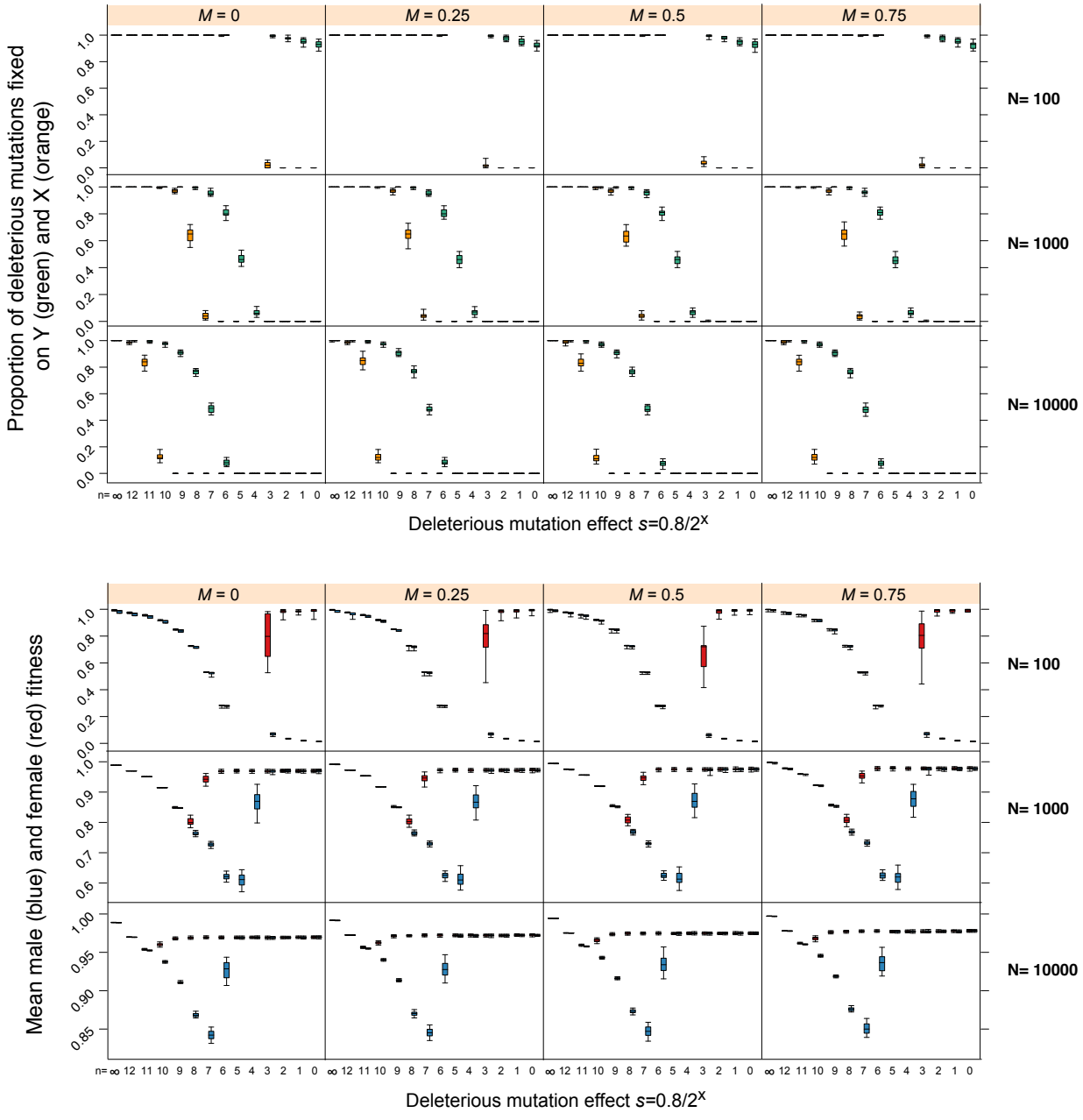


Figure S4. A) Equilibrium proportions of deleterious mutations fixed on X (orange boxes) and Y (green boxes) and B) equilibrium fitness values for females (red boxes) and males (blue boxes) for varying strength of deleterious mutation selection (s = 0 to 0.8, logarithmic scale on horizontal axis, with $s=0.8/2^n$, where n = (0, 1,.. 12 and ∞). The shift from accumulation to purge occurs at lower s values for the X than for the Y, and also for large than for small populations (N =100 to 10'000), but sex-antagonistic selection had no effect (M = 0 to 0.75). Other parameter values σ_E^2 =0 and μ=10^{-4}. Missing values at intermediate s values for N=100 are due to extinctions. Box plots as in Figure 3.



CHAPTER V

EXTINCTION RISKS UNDER CLIMATIC CHANGES: WHAT ROLE FOR SEX-DETERMINATION SYSTEMS?

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ABSTRACT

Ongoing climatic changes are expected to threaten species with temperature-dependent sex determination (TSD) by biasing population sex ratios. In contrast, species with genotypic sex determination (GSD) are implicitly assumed immune against such changes, because genetic systems are thought to necessarily produce even sex ratios. These contrasting expectations rely on the classical view that TSD and GSD proceed from fundamentally different and mutually exclusive mechanisms. Recent molecular advances, however, are revealing a striking uniformity of sex-determination pathways across vertebrates; TSD and GSD are better seen as the two ends of a continuum. We formalize this continuum in a quantitative-genetics framework and parameterize norms of reaction from published datasets, in order to quantify sex-ratio sensitivity to temperature in a wide range of systems. As this formalization shows, expected climatic changes have the potential to bias sex ratios also in species displaying GSD under current conditions. As such species have not been challenged by sex-ratio selection over recent evolutionary times, they may also lack the behavioral plasticity evolved by TSD species, as well as the genetic variance in reaction norms required for a quick evolutionary response. Hence, sex-ratio biases by climatic changes may represent a previously unrecognized extinction threat also for some GSD species.

INTRODUCTION

Tuataras are living fossils, the only surviving members of the order Sphenodontia, now restricted to small islands off New Zealand. The few remaining populations have survived extinction by man-introduced rats, but are now facing a new, potentially lethal threat, caused by climatic changes: Their sex is determined by environment, sex ratios becoming male-biased as temperature increases. Given the present trend, clutches are expected to produce 100% males at the current nest sites by the year 2080 (Mitchell et al. 2008). Being unable to swim long distances, they will not have the opportunity to colonize cooler habitats. Tuataras might thus be bound to extinction due to their temperature-dependent sex determination.

Rising temperatures are generally expected to constitute a serious threat not just for tuataras, but for all species with temperature-dependent sex determination (TSD), as now addressed in an increasing number of studies (Janzen 1994; Robert & Thompson 2001; Morjan 2003; Janzen & Phillips 2006; Hawkes et al. 2007; Hulin et al. 2009; Mrosovsky & Provancha 2009; Witt et al. 2010; Fuentes et al. 2010; Mitchell & Janzen 2010). In sharp contrast, species with genotypic sex determination (GSD) are implicitly assumed immune against such risks, because GSD is generally expected to necessarily produce even sex ratios.

Such contrasting expectations rely on the classical dichotomous view of sex determination, which sees TSD and GSD as fundamentally different processes, underlain by mutually exclusive mechanisms (Valenzuela et al. 2003). Ongoing molecular work, however, is now revealing a striking uniformity of sex determination pathways across vertebrates (Graves & Peichel 2010), raising the alternative view that GSD and TSD actually represent two ends of a continuum (Sarre et al. 2004). Thus, if TSD and GSD indeed rely on fundamentally similar mechanisms, then the question has to be asked, whether rising temperatures may also constitute a threat for species that display genotypic sex determination under current conditions.

In this review, we will briefly outline the role of temperature in sex-determination across vertebrates, then present arguments in favor of the alternative view (sex determination as a continuum). We will then argue that quantitative genetics provides a unifying framework to formalize this continuum, accounting for both the diversity of sex-determination patterns across vertebrates, and the frequency of transitions among them. A key concept here is that of reaction norm, which expresses the amount of sex-determination factors produced by a given genotype as a function of environment.

To illustrate this view, we will parameterize norms of reaction from series of published data sets of temperature-dependent sex ratios, and from these parameters evaluate sex-ratio sensitivities to temperature

changes. How such sensitivities should translate into extinction risks will obviously depend on other environmental factors and species characteristics (e.g. habitat fragmentation, mating system, or standing genetic variance for reaction norms). However, one noticeable outcome of our review is that some species displaying GSD under current conditions may well be less armed to face climatic changes than are many TSD species.

SEX DETERMINATION IN VERTEBRATES

Like other mammals, humans display genetic sex determination. The mammalian master sex-determining gene (*SRY*) appeared on a proto Y chromosome some 170 million years ago, and has since kept its function in all marsupials and eutherians, except for a few rodents (Graves 2008). Recombination then progressively stopped in males, favored by epistatic interactions with sex-antagonistic genes (Rice 1996). As a side effect, deleterious mutations progressively accumulated on the non-recombining Y chromosome (Bergero & Charlesworth 2009), with the result that mammalian sex chromosomes are now highly differentiated, with a strongly decayed Y chromosome in males (the so-called heterogametic sex). The very same process occurred in birds, except that females are the heterogametic sex, and generally carry a decayed W chromosome. There is no evidence in either group that temperature affects sex determination under natural conditions. Sex ratios do sometimes vary with temperature (Goth & Booth 2005), but due to differential embryonic mortality (Eiby, Wilmer, & Booth 2008).

These features are in fact remarkable when placed in a broader context. The sex chromosomes found in ectothermic vertebrates (fish, amphibians, reptiles, in which body temperature depends on environment) are usually poorly differentiated and display high turnover rates (Devlin & Nagahama 2002; Eggert 2004; Ezaz, Sarre, et al. 2009a; Ezaz, Quinn, et al. 2009b; Pokorna & Kratochvil 2009; Organ et al. 2009;). In many cases, such chromosomes are simply lacking, and sex is determined solely by environment.

Temperature dependence was first evidenced in reptiles (Charnier 1966), where it has now been found in far more than 100 species (Valenzuela & Lance 2004; Ezaz, Sarre, et al. 2009a; Pokorna & Kratochvil 2009; Gamble 2010), though not randomly regarding phylogeny. Sex determination is only genotypic in snakes, which often present heteromorphic sex chromosomes and female heterogamety (Olmo 1986), whereas temperature is normally the main or only factor in crocodilians and sphenodontia (Harlow 2004, Deeming 2004). TSD is also common in turtles, but genetic sex determination, with both male and female heterogamety has been found in nearly 20% of the species investigated (Table S2 of this ms; e.g. Ezaz, Valenzuela, et al. 2006b; Kawai et al. 2007;

Martinez et al. 2008). Lizards present a wide diversity of systems, with differentiated sex chromosomes found in about 20% of species karyotyped (Olmo 1986; Ezaz, Sarre, et al. 2009a; Gamble 2010). Patterns also show some clustering at this scale, with e.g. frequent TSD in agamids but GSD in the ecologically similar iguanids (Pokorna & Kratochvil 2009). Phylogenetic distribution suggests multiple independent evolutionary origins and frequent transitions among sex determination systems, sometimes within genera or even within species (Ezaz, Sarre, et al. 2009a; Pokorna & Kratochvil 2009; Gamble 2010). Both TSD and GSD, for instance, occur in *Ctenophorus* and *Amphibolurus* (Harlow 2004) as well as in geckos, with male or female heterogamety (Gamble 2010). In the snow skink *Niveoscincus ocellatus*, TSD occurs in lowland and GSD in highland populations (Pen et al. 2010). TSD may also co-occur with GSD (mixed sex determination system, where genes and environment interact to determine sex), even when sex chromosomes are differentiated, such as in the skink *Bassiana duperreyi* (Radder et al. 2008), the dragon lizard *Pogona vitticeps* (Ezaz et al. 2005; Quinn et al. 2007) or the Japanese gecko *Gekko japonicus* (though possibly in different populations; Gamble 2010).

Very similar patterns have been shown to occur in fish. Sex chromosomes are differentiated in less than 10% of the species investigated (Devlin & Nagahama 2002), and temperature dependence has been found in more than 60 species, among which 30 ssp of *Apistogramma* cichlids (Romer & Beisenherz 1996; Ospina-Álvarez & Piferrer 2008). GSD and TSD have also been shown to coexist in species of Atherinopsidae (*Menidia:* Conover & Kynard 1981) and Cichlidae (*Oreochromis:* Baroiller et al. 2009a).

In amphibians, surprisingly, only GSD seems to prevail in nature, but less than 4% of species karyotyped show differentiated sex chromosomes (Eggert 2004). Furthermore, sex is easily reversed in this group (as in many other ectothermic vertebrates) by temperature conditions that depart only slightly from natural conditions (Dournon et al. 1990).

The full range of TSD among ectothermic vertebrates is not yet known: few species have been investigated so far (especially among fish and amphibians), and some studies only considered narrow temperature ranges. (Strussmann et al. 1996) described pure GSD for pejerrey *Odontesthes* (=*Patagonina*) *hatcheri*, but later found thermolability (Strussmann et al. 1997). The dragon lizard *Pogona vitticeps* was first claimed to have GSD (Viets et al. 1994) but then revealed a mixed system (where genes and environment interact to determine sex, (Quinn et al. 2007). Similarly, Viets et al. (Viets et al. 1993) found female leopard gecko (*Eublepharis macularius*) to be produced not only at low (as previously thought), but also high temperatures, with males in between. The temperatures required for sex reversal might actually be close to lethal for some species, while sex-biased mortality can mistakenly be interpreted as TSD. By and large, it is likely that

temperature will be found to play a role in sex determination in many more fish and reptiles, and possibly amphibians.

WHY HAVING TSD?

Temperature effects are not always adaptive. In many instances, shifts in sex ratios may only express underlying metabolic or regulatory constraints. This is certainly the case for species in which sex reversal only occurs outside the natural temperature range (such as found among amphibians). All metabolic processes in ectotherms depend on external temperature, and, as further developed below, this also applies to gene expression and enzymatic activity along the sex-determination cascade. The stability of GSD among birds and mammals (as opposed to the lability found among ectotherms) does not result from intrinsic differences in metabolic pathways, but rather from the ability of endotherms to control temperature during the sensitive period of embryonic development, when the phenotypic sex is determined.

The question of adaptive significance arises for species in which sex is affected within the natural temperature range. This question has often been addressed (Charnov & Bull 1977; Janzen & Phillips 2006; Freedberg & Taylor 2007; Warner & Shine 2008; Chandler, Phillips, & Janzen 2009; Warner, Uller, & Shine 2009), but responses are not always straightforward. It is worth noting that temperature often affects phenotypic traits other than sex (Rhen & Lang 2004). If such traits have sex-specific relations to fitness, then sexes differ in their optimal temperature for development, which should select for TSD (Charnov & Bull 1977). Sex-specific incubation temperatures in the agamid *Amphibolurus muricatus*, for instance, are also those maximizing sex-specific reproductive success (Warner & Shine 2008, though the picture becomes more complex with fluctuating temperatures, Warner & Shine 2011). In short-lived (annual) species, earlier birth increases the opportunity for growth, which may boost fecundity in females more than in males. This has selected for TSD in *Menidia* (Conover 1984) and *Niveoscincus* (Pen et al. 2010), where females are produced earlier in the season.

GSD normally produces balanced sex ratios, corresponding to the optimal Fisherian sex ratio in panmictic, freely mixing populations. TSD by contrast introduces potential biases, which may seem an important drawback. However, TSD species also display behavioral and physiological adaptations (as for instance nest site choice:(Doody et al. 2006;), which potentially allow maternal control of sex ratios. Nest-site choice in oviparous species is often elaborate enough that individual females produce mixed clutches via the temperature gradient within nests (as in tuataras, Thompson et al. 1996)). Viviparous species may control sex ratios by adjusting

basking opportunities (*Niveoscincus ocellatus*, Wapstra et al. 2004), and mouth-breeders by shifting among specific microenvironments (e.g. the tilapia *Oreochromis,* Baroiller et al. 2009a). TSD might actually confer selective advantages over GSD whenever optimal sex ratios differ from even, as may happen under local mate competition.

ARE GSD AND TSD QUALITATIVELY DISTINCT?

As pointed out in the introduction, sex determination is often seen as a dichotomous process, where individual sex is determined either by its genotype, or by its environment. As formulated by (Valenzuela et al. 2003), these represent two distinct and mutually exclusive mechanisms: In GSD, individual sex is determined at fertilization by genes, whereas in TSD, sex is determined after fertilization by temperature (Valenzuela et al. 2003; Ospina-Alvarez & Piferrer 2008, Pokorna & Kratochvil 2009).

A contrasting view sees GSD and TSD as the two ends of a continuum (Sarre et al. 2004; Grossen et al. 2011). Sex-determination pathways are highly conserved in vertebrates, including both GSD and TSD species, with the same genes active along the cascade (Graves & Peichel 2010). Evolutionary transitions usually involve only master genes, at the top of the cascade (Volff et al. 2007). Temperature may interfere at many stages by influencing the activity of enzymes (such as aromatase, which converts testosterone into estradiol, or reductase, which converts testosterone into non-aromatazible dihydrotestosterone, Crews & Bergeron 1994; Baroiller, Guiguen, & Fostier 1999; Lance 2009; Nakamura 2009) or the expression of genes (such as DMRT1, see below). In *Oreochromis niloticus*, for instance, high temperatures (35°C) repress *FoxL2* and *Cyp19* (involved in aromatase production: Wang et al. 2007; Lance 2009; Ramsey & Crews 2009), and up-regulate the masculinizing genes *Amh* and *Sox9*, so that *XX* individuals develop into males (Baroiller et al. 2009a).

New sex chromosomes recently evolved in the medaka fish (*Oryzias latipes*) through the duplication of *DMRT1*. At normal temperatures (25°C), *DMRT1* expression from the autosomal copy is too low to reach the threshold required to induce male development, so that *XX* individuals develop into females. By contrast, *XY* individuals develop into males due to the additional expression of the duplicated copy on the proto Y (*DMRT1bY*). Higher temperatures (32°C), however, up-regulate the autosomal *DMRT1* copy, so that a significant fraction of *XX* individuals develop into males (Sato et al. 2005; Hattori et al. 2007). Sex is thus determined by the amount of the *DMRT1* transcription factor, which itself depends on a combination of genetic and environmental effects.

In line with this alternative view, (Grossen et al. 2011) proposed to define sex-determination systems at the population (not individual) level, as the proportion of variance in phenotypic sex stemming from genetic versus environmental causes. In GSD, all or most of this variance can be assigned to genetic factors, whereas in TSD, all or most can be assigned to environmental factors (temperature). In between are mixed systems such as described above, where genes and environment interact to determine sex.

THRESHOLD TRAITS AND NORMS OF REACTION

In this quantitative-genetics framework, sex can be seen as a threshold trait (Bull 1981; Bulmer & Bull 1982; Roff 1996; van Dooren & Leimar 2003; Sarre et al. 2004; Quinn et al. 2007; Pen et al. 2010) underlain by a liability factor *A* (sex-determining factor, such as *DMRT1* expression in medaka). Any individual will develop into a male if its trait value *A* exceeds a threshold ξ, and into a female otherwise (see Appendix).

Gene-environment interplay can be formalized as a norm of reaction, which quantifies the phenotype (*A*) expressed by a genotype (*IJ*) as a function of environment (*T*). Such norms can be linear (with positive or negative slope), or non-linear (e.g., quadratic). Different genotypes express different norms (due to genes with major or minor effects), possibly with different shapes (gene-environment interactions). Genetic variance within populations arises from the coexistence of different genotypes, and environmental variance from microenvironments: different individuals within a population or clutch may experience different temperatures (both in terms of average and fluctuations) during the sensitive embryonic period.

Temperature effects originate from the laws of thermodynamics: as pointed out above, the kinetic energy of physico-chemical processes underlying sex determination depends on temperature. GSD species have evolved independence from such effects by relying on major-effect alleles (coexistence of two or more genotypes coding for liability trait values far apart from the threshold), so that sex only depends on genotype under natural temperature ranges. Genotype frequencies and sex ratios (here defined as the proportion of males in a population) are maintained balanced by frequency dependence. By contrast, TSD species have capitalized on these underlying constraints to evolve temperature dependence as an adaptive strategy. Besides the behavioral and physiological co-adaptations already mentioned, this implied local fine-tuning of reaction norms.

Sex expression of given genotypes across a temperature range (i.e., norms of reaction) can be investigated from the variable sex ratios produced by single or mixed genotypes under a range of temperature

values (see Appendix). To illustrate this approach, we collected 65 data sets from the literature and evaluated parameters of linear and quadratic norms fitted to either pure TSD or mixed systems. Our Supplementary Material lists these data sets and the model fitted including parameter estimates. This list is not intended to be exhaustive, but to illustrate our review.

Pure TSD

Pure TSD refers to the situation where a single genotype is fixed at the sex- determining locus, so that phenotypic variance only stems from environmental effects. From our literature survey, 29 data sets (27 species) could be fitted as linear TSD (Fig.1). In 22 species, increasing temperatures induced female-biased sex ratios (MF patterns, Fig. 1a-b), with β values (standardized slope) between -5.1 °C^{-1} (Pond slider *Trachemys scripta*) and -0.36 °C^{-1} (Yellow Mud Turtle *Kinosternon flavescens*). Five other species showed the opposite FM pattern, with a sex ratio increasing with temperature (Fig. 1c). Values for β also varied by more than one order of magnitude, from 0.12 °C^{-1} in the Jacky dragon (*Amphibolurus muricatus)* to 3.28 °C^{-1} in tuatara (*Sphenodon punctatus*). Pivotal temperatures (i.e., the temperature producing even sex ratios) throughout linear TSD systems varied from 14.6°C (Atlantic silverside *Menidia menidia*) to 34°C (*Amphibolurus muricatus*).

Curvilinear norms are expected whenever temperature has conflicting effects on the liability trait (e.g. boosting activity of both aromatase and aromatase inhibitors, (Girondot et al. 2010)). Sex ratios are then non-monotonic functions of temperature, with two pivotal temperatures left and right of the vertex (temperature at which sex ratio is maximal). We found pure TSD with a curvilinear norm in 25 sets (23 species). In all of them, females were produced at both low and high temperatures, and males in between (FMF pattern; Fig. 1d). Estimates for γ (steepness of the curve) ranged from -2.97 °C^{-2} (mugger crocodile *Crocodylus palustris*) to -0.08 °C^{-2} (three-striped Mud Turtle *Kinosternon baurii*).

Mixed systems

In the simplest case, mixed sex-determination systems result from two alleles (e.g. *X* and *Y*) with major effects (i.e., large differences in associated liability trait values within the normal temperature range) and parallel reaction norms. As the two genotypes (*XX* and *XY*) may show highly divergent pivotal temperatures, fitting the full model requires data for a large range of temperature values. We could fit nine data sets comprising a minimum of five different temperatures. In some instances this included the two pivotal temperatures (e.g. Fig 2 a-b), but in others we could only fit one genotype, raised either independently (e.g. *XX* in the goldfish *Carassius auratus*, Fig. 2c) or in combination (e.g. *ZZ* in the dragon lizard *Pogona vitticeps*, Fig. 2d). The range of β values

was also quite large, from -5.17 in the Central Bearded Dragon (*Pogona vitticeps*) to 0.29 in the crested newt *Triturus cristatus cristatus*.

A curvilinear mixed system was only found in the flatfish hirame (*Paralichtys olivaceus*, Fig. 2e). Only the *XX* genotype could be adjusted (*XY* always produced males within the range investigated), with a positive γ value (0.03 $°C^{-2}$), inducing a MFM pattern. A curvilinear mixed system was also fitted by Quinn et al. (2007) to *Pogona vitticeps* data, but we found these more parsimoniously explained with linear norms. Curvilinear mixed systems are certainly more widespread in nature, but require high quality empirical data, since four parameters have to be estimated.

Variance within and between populations

Genetic polymorphism for genes with major or minor effects can be found both within and among populations. A classical example is provided by Atlantic silversides (*Menidia menidia*), in which sex determination varies from pure GSD at lower latitudes to strong TSD at high latitudes (Conover & Heins 1987; Lagomorsino & Conover 1993). A similar pattern exists in *Niveoscincus ocellatus*, with a GSD system in lowland and TSD in highland populations (Pen et al. 2010). Laboratory strains of the fish *Poeciliopsis lucida* may also show either GSD or TSD (Sullivan & Schultz 1986), but the latter might have evolved under laboratory conditions through the loss of one major-effect allele by drift or selection (e.g. loss of the *Y* allele, if strains are reared close to the *XX* pivotal temperature).

Another striking example for latitudinal trends in temperature-dependence comes from American snapping turtles (*Chelydra serpentina*). As shown by Ewert et al. (2005), populations from five different latitudes all share a FMF pattern with a rather constant vertex (T_m), but the range defined by the two pivotal temperatures broadens with latitude. This pattern can be fitted by a family of quadratic functions with variable T_m, γ and k, where k accounts for most of the variance (Fig. 3a).

Within-population variance has also been documented, e.g. for pivotal temperatures in turtles (see e.g. refs, Janzen 1992; Rhen & Lang 1998; Dodd, Murdock, & Wibbels 2006) or k parameters in alligators (Rhen & Lang 1998). We fitted data from Baras et al. (2001) on the male-heterogametic tilapia *Oreochromis niloticus*, showing that only *XX* individuals were sex-reversed within the range investigated. Norms were linear increasing, with among-family variance in both the slope and pivotal temperature (Fig. 3b). Within-populations segregation of small-effect alleles was also described in *Menidia* (Conover & Heins 1987).

THREATS FOR TSD AND GSD SPECIES DUE TO CLIMATIC CHANGES

TSD species have long been recognized to be vulnerable to temperature changes. More than 15 years ago, Janzen (1994) cautioned that painted turtles *Chrysemys picta* might be unable to evolve fast enough in response to ongoing climatic changes. Average air temperature is expected to increase by 1.1°C to 6.4°C by the end of the 20th century (IPCC Fourth Assessment Report). Even though nest substrate as well as sun exposure have important additional effects (De Souza & Vogt 1994), nest temperatures have been shown to correlate with air temperature in turtles (Janzen 1994; Glen & Mrosovsky 2004): *Erethmochelys imbricata*) and lizards (Telemeco, Elphick, & Shine 2009): *Bassiana duppereyi*). Parameterization of reaction norms, as proposed here, should allow evaluating the sensitivity of sex ratios to temperature changes across a variety of sex-determination systems, including both TSD and GSD.

From the values collected here, climatic changes should indeed have serious impact on several TSD species. Hawkbill turtles (*Eretmochelys imbricata*, Fig. 1a), for instance, show high sex-ratio sensitivity to temperature ($\frac{\beta}{\sqrt{2\pi}}$ =-1.57°C^{-1}, see Appendix). An increase in nest temperature by only 0.1°C would lower sex ratio from 0.5 to about 0.34, and a 1.1 °C increase would eradicate males. Even stronger sensitivity (-2.04°C^{-1}) is estimated for the Pond slider *Trachemys scripta*. Sex ratios in green turtles (*Chelonia mydas*, Fig. 1b) display much lower sensitivity (-0.47°C^{-1}), but would still drop to about 0.1 for a 1.1°C increase.

Species displaying GSD under normal conditions are often considered immune against such changes, because genetic systems are assumed to necessarily produce even sex ratios. However, in line with the formalization provided here, sex-ratio biases might accrue at temperatures only slightly outside the natural range. Assuming linear norms (the model with strongest empirical support), sex ratios are somewhat buffered against changes in between the two pivotal temperatures ($\tilde{T}_2 - \tilde{T}_1$), being adjusted by changes in Y frequency (Grossen et al. 2011). Therefore, as long as XY females and XX males are viable and fertile, temperature will little affect sex ratios in species with a large interpivotal range ($\tilde{T}_2 - \tilde{T}_1$). When getting close to pivotal temperatures, β values become relevant. Sex ratios will then change abruptly for steep slopes (Fig. 2b), more smoothly and continuously for shallow slopes (Fig. 2a; see also Fig.4 in Grossen et al. 2011).

P. reticulata (Fig. 2a), for instance, has a relatively low sex-ratio sensitivity to temperature (0.10°C^{-1}). Starting from even at the midpoint between the two pivotal temperatures (~25°C), sex ratio can be maintained at 0.5 for a temperature increase of 1.1°C by decreasing the frequency of the Y. An increase by 6.4°C, however is

expected to lead to nearly 80% males. Note that sex-biased mortality could not be ruled out for *P.reticulata* (Karayucel et al. 2006). *P. hatcheri* (Fig. 2b) has a similar $\tilde{T}_2 - \tilde{T}_1$ range, but shows a higher sensitivity (0.41°C^{-1}). No bias is expected for a 1.1°C increase, but 100% males are predicted for the worst scenario. By contrast, *Bassiana dupperreyi*, has a slightly larger $\tilde{T}_2 - \tilde{T}_1$ range and displays lower sensitivity (-0.06°C^{-1}). It is thus expected to resist better to climatic changes. No shift in sex ratio is expected, even if nest temperatures increase by 6.4°C. Note that the calculations above assume temperature changes being smooth enough that sex ratios can be controlled within the inter-pivotal range by adjusting the frequency of Y (or W) chromosomes. For a mixed system, the sensitivity values provided above are therefore expected to apply once pivotal temperatures are reached (see Fig.4 in Grossen et al. 2011). Abrupt temperature changes and/or severe fitness reduction of sex-reversed individuals will increase the risk of sex-ratio biases.

INTERACTING FACTORS

Besides sensitivity to temperature changes, several important factors will interact to accelerate or slow down population or species extinctions (Mitchell & Janzen 2010). These factors include demography, behavior, ecological responses, and evolutionary transitions between sex determination systems.

Demographic aspects include the population-dynamics consequences of sex-ratio changes. Sex-ratio biases are normally opposed by Fisherian sex ratio selection, but may still have positive impact on population dynamics. Except under strict monogamy, female bias may first boost population growth (Rankin & Kokko 2007). This scenario is expected in many reptiles, as a temperature increase would generally lead to female-biased sex ratios (Freedberg & Taylor 2007; Kallimanis 2009). Fish and amphibians, by contrast, often show male biases with increased temperatures (Table S2) and will therefore be more at risk. In both cases, however, biased sex ratios lead to a decreased effective population size, whereby genetic drift is increased.

Climatic changes involve not only increased average temperatures, but also increased fluctuations. Longevity and overlapping generations may be important to stabilize sex ratios over time. Many reptiles with TSD are long-lived. Tuataras first breed at age 15 and lay three to eight eggs every nine years (Mitchell et al. 2010). High longevities are also found in turtles, and may similarly buffer against demographic stochasticity. However, long generation times and low birth rates will also slow down genetic adaptation to temperature changes.

Behavioral responses matter by allowing spatial or temporal shifts in activity. Lighter sand beaches, for instance, might become more important to sustain marine turtle populations, by providing cooler nest sites (Hays et al. 2003). Reduced basking may lower predation risk in viviparous TSD species. Several studies have already documented earlier breeding in oviparous TSD species in response to climate change (Weishampel, Bagley, & Ehrhart 2004; Zhang et al. 2009). Over 19 years, oviposition season was advanced by an estimate of 10 days in Chinese alligators (Zhang et al. 2009). Thus, TSD species may have already evolved behavioral adaptations allowing fine-tuning of sex ratios to local conditions. Species displaying pure GSD within historical temperature ranges are not expected to harbor such adaptations, and might therefore be more at risk once pivotal temperatures are reached.

Habitat shifts seem an obvious response to climatic changes. However, habitat fragmentation and physical constraints will often limit the possibility of species to move to cooler habitats. In the case of island-inhabiting tuataras, translocations might be required if the species is to be conserved (Mitchell et al. 2010). Though tuataras might be saved, human interventions are not feasible at large scale.

Sex-ratio selection induced by climatic changes may also trigger shifts in sex determination systems. Individual-based simulations by Grossen et al. (2011) predicted not only extinctions, but also transitions among alternative sex-determination systems, depending on mutation rate, effective population size, and strength of the climatic trend. The spread of new reaction norms (with e.g. shifted pivotal temperatures) also fostered turnovers in sex-determination mechanisms. As a matter of fact, the large diversity of SD systems, frequent transitions, and prevalence of homomorphic sex chromosomes found among ectothermic vertebrates likely reveals a history of repeated adaptation to new temperatures, triggered by climatic changes or range expansions. Frequent transitions might actually help maintaining evolutionary potentials. The simulation study by Grossen et al. (2011) showed that species with differentiated sex chromosomes (resulting from the decay of non-recombining Y chromosomes during evolutionary stasis) suffered more from extinctions under climatic changes.

As outlined above, local adaptation of sex-determination systems has been established both in fish (Conover & Kynard 1981) and reptiles (Pen et al. 2010), where populations display either TSD or GSD depending on local conditions. Whenever searched for, genetic variance in reaction norm has been found in TSD systems, both within and among populations. Genes underlying nesting behaviors have also shown polymorphism (e.g. McGaugh et al. 2010 in *Chrysemys picta*). Variance is presumably maintained among populations by local adaptation to different environments, and within populations by temporal fluctuations in selective regimes and dispersal.

Standing genetic variation for traits relating to sex ratios will enable quick adaptive responses to selection. Laboratory populations of Atlantic silversides (*Menidia menidia*) subjected to Fisherian sex-ratio selection have evolved from biased to equal sex ratio in less than eight generations (Conover & Vanvoorhees 1990). Heritability of thermo-sensitivity and rapid response to sex-ratio selection was also found in Nile tilapias (Wessels & Hoerstgen-Schwark 2007). In the rainbow trout *Oncorhynchus mykiss* (a species with mixed sex determination; Table S2) one generation of directional selection was enough to generate significant changes in sex ratio, with heritability estimates ranging from 0.63 to 0.71 (Magerhans & Hoerstgen-Schwark 2010).

CONCLUSIONS

Contrasting with the conventional dichotomous view of sex determination, quantitative genetics provides a unifying perspective that allows better accounting for the sex-determination patterns found in ectothermic vertebrates, including coexistence of different systems, frequent transitions among them, and prevalence of homomorphic sex chromosomes. Formalization in terms of reaction norms furthermore allows quantifying the shifts in sex ratio expected under temperature changes.

Are TSD species really at risk? On the one hand, such species have a history of adaptation to temperature changes, and a large potential to further adapt through behavioral plasticity or genetic variance. Ancient TSD clades have persisted for more than 200 million years of cycles of cooling and warming. On the other hand, however, we do not know how many TSD species went extinct due to climatic changes (TSD has been suggested to play a role in dinosaur extinctions, Miller 2004). The current rise in temperature is very abrupt and probably too fast for many species to adapt. In *Sphenodon punctatus*, highly male-biased sex ratios (0.65) already occur during warm years (Harlow 2004). Offspring sex ratios in painted turtle (*Chrysemys picta*) also display strong correlations with mean July air temperature (Janzen 1994). Given the sex-ratio sensitivities to temperature obtained here, there is no doubt that climatic changes will threaten some TSD species.

What about mixed and GSD systems? The quantitative-genetics perspective also underlines the strong similarities between GSD and TSD mechanisms, which differ in no fundamental way but rather represent the two ends of a continuum (Sarre et al. 2004). It follows from our formalization that a range of species displaying pure GSD under current conditions might equally be at risk. Parameters estimates for norms of reaction also predict in some cases strong sensitivity to expected temperature changes. Highly biased sex ratios have already been found in natural populations of fish (Nagler et al. 2001) and frogs (Matsuba et al. 2008) that are normally

considered pure GSD. Such species have not been challenged by sex-ratio selection for a long time, and may thus lack the behavioral plasticity evolved by TSD species. For the same reason, they may also lack the among- or within-population genetic variance in reaction norms required for quick evolutionary response. Climatic changes may thus represent a previously unrecognized extinction threat also for some GSD species.

APPENDIX: FORMALIZING TEMPERATURE DEPENDENCE IN SEX DETERMINATION.

Several formalizations of temperature effects have been proposed, focused on pure TSD species. These models directly fit sex ratios (a sigmoid response variable constrained between 0 and 1). Girondot (1999) for instance used a logistic function with two parameters (slope and inflection point), fitted to either MF or FM patterns. Additional parameters were later introduced when fitting a variety of systems in turtles (Godfrey, Delmas, & Girondot 2003; Hulin et al. 2009), to account for asymmetries in the sigmoid function and non-monotonic (FMF) patterns. Sigmoid functions were similarly used in simulation studies to investigate the evolution of sex determination and condition-dependent sex allocation in fluctuating environments (Schwanz & Janzen 2008; Schwanz, Janzen, & Proulx 2010).

We choose instead to fit sex ratios as explicit function of linear or quadratic reaction norms for the liability trait. As the aim of our study was to compare a broad range of species and a variety of sex determination systems, we opted for a parsimonious approach relying on few, easy-to-estimate parameters, and allowing direct comparison between pure TSD and mixed systems. We certainly made simplifications (e.g. assuming curvilinear norms to be symmetric), but the very good fits obtained suggest these simplifications to be acceptable.

We caution, however, that the fitted data sets in most cases result from constant-temperature laboratory experiments, whereas temperatures fluctuate in nature, which might also affect outcomes (Bull 1985). Specific models have been proposed to account for such fluctuations, including mechanistic models of TSD (Georges, Limpus, & Stoutjesdijk 1994; Valenzuela, Botero, & Martinez 1997; Georges et al. 2004; Delmas et al. 2008; Girondot et al. 2010; Warner & Shine 2011), but appear too complex to be applicable outside very specific and precisely documented situations. Though species-specific models are certainly needed to provide testable predictions, as done for instance for a few case studies in turtles (e.g. Hawkes et al. 2007; Fuentes et al. 2010) and tuatara (Mitchell et al. 2008; 2010), we instead opted for a simple and general approach readily applicable to a variety of systems.

We thus formalized temperature effects in a quantitative-genetics framework, where sex is a threshold trait, underlain by a liability factor (see e.g. Rhen & Lang 1998; Quinn et al. 2007; Pen et al. 2010 for similar conceptualizations). Individual liability trait values depend on genotype *IJ*, mean local temperature *T*, and individual deviation from this mean (stemming from the micro-environment experienced during the sensitive period of embryonic development). This within-population environmental variance induces a distribution around the mean trait value $A_{IJ,T}$ (expectation for genotype *IJ* at temperature *T*), which we assume normal with

standard deviation σ_E. Any genotype *IJ* may thus produce both males and females, depending on how close to the threshold ξ lies $A_{IJ,T}$, in units of σ_E. The relevant quantity to calculate the sex ratio (proportion of males) produced by genotype *IJ* at temperature *T* is thus its standardized liability trait $\alpha_{IJ,T} = \frac{A_{IJ,T} - \xi}{\sigma_E}$, a dimensionless quantity. Sex ratio ($r_{IJ,T}$, as proportion of males) is then obtained as the area of the normal probability density function above the threshold (Grossen et al. 2011):

$$r_{IJ,T} = \frac{1}{2}\left(1 + erf\left(\frac{\alpha_{IJ,T}}{\sqrt{2\pi}}\right)\right),$$

where *erf* is the so called error function. When several genotypes segregate, population sex ratio is calculated over the several density functions. E.g., assuming two genotypes *XX* and *XY* with equal frequencies:

$$r_T = \frac{1}{4}\left(2 + erf\left(\frac{\alpha_{XX,T}}{\sqrt{2\pi}}\right) + erf\left(\frac{\alpha_{XY,T}}{\sqrt{2\pi}}\right)\right).$$

For linear norms, the standardized liability trait was modeled as $\alpha_{IJ,T} = \beta\left(T - \tilde{T}_{IJ}\right)$, where β measures the standardized slope (increase in standardized liability factor per unit increase in temperature, units °C^{-1}) and $\tilde{T}_{IJ}$ the pivotal temperature for genotype *IJ* (i.e. the temperature at which this genotype provides an even sex ratio). In a linear TSD, sex ratio becomes a sigmoid function of temperature, with an inflection point at the pivotal temperature (which is expected to match local average temperature, due to sex-ratio selection). The sensitivity of sex ratio to temperature change at this point is given by $\frac{dr}{d\alpha}\frac{d\alpha}{dT} = \frac{\beta}{\sqrt{2\pi}}$.

Curvilinear norms were fitted with quadratic functions, expressed as $\alpha_{IJ,T} = \gamma(T - T_m)^2 + k$ in a standard (vertex) form, or equivalently $\alpha_{IJ,T} = \gamma(T - \tilde{T}_1)(T - \tilde{T}_2)$ in a factor form, where γ measures the steepness of the parabola (in units of °C^{-2}), $\tilde{T}_1$ and $\tilde{T}_2$ are the pivotal temperatures, and $T_m = \frac{\tilde{T}_1 + \tilde{T}_2}{2}$ defines the vertex (temperature at which the liability trait is maximal). The temperature sensitivity of the standardized liability trait α can also be calculated at the pivotal temperatures as $\gamma\left(\tilde{T}_1 - \tilde{T}_2\right)$ and $\gamma\left(\tilde{T}_2 - \tilde{T}_1\right)$ respectively. The sensitivity of sex ratio to temperature change at these points is given by $\frac{dr}{d\alpha}\frac{d\alpha}{dT} = \frac{\gamma\left(\tilde{T}_1 - \tilde{T}_2\right)}{\sqrt{2\pi}}$ and $\frac{\gamma\left(\tilde{T}_2 - \tilde{T}_1\right)}{\sqrt{2\pi}}$ respectively.

All data fits were performed in R (R Development Core Team). Parameters per species data set (Tables S1a-d) were estimated with the nonlinear least squares option *nls* (package stats), using sample size as weight

when available. For model selection, we used the Akaike Information Criterion (function AIC in package stats), corrected for small sample sizes (AICc) according to Burnham and Anderson (2004). To fit families of curves for *Chelydra serpentina* populations and *Oreochromis niloticus* families (Tables S1e,f), nested series of binomial models were fitted by maximum likelihood, using the function *mle2* (package bbmle). The fitted functions were of the form $numberOfMales \sim dbinom(prob = f(), size = totalNumber)$ where $f(\gamma, T_m, k) = pnorm(\gamma \cdot (temperature - T_m)^2 + k)$ in the case of *Chelydra* and $f(\beta, \tilde{T}_{XX}) = \frac{1}{2} \cdot (1 + pnorm(\beta \cdot (temperature - \tilde{T}_{XX}))$ for *Oreochromis.* We specified with the option *parameters*, which parameters differed between groups for a certain model. Comparisons between nested models were performed using Likelihood Ratio Tests.

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FIGURES

Figure 1. *Pure TSD.* Right panels: sex ratio as a function of temperature (observed points and fitted model). The horizontal dotted line (r=0.5) defines the pivotal temperature ($\tilde{T}_{IJ}$, arrows). Left panels: corresponding reaction norms with standardized normal distribution (blue curve) and threshold (horizontal dashed line). a) *Eretmochelys imbricata* (Godfrey et al. 1999), linear fit, negative β with steep slope. b) *Chelonia mydas* (Spotila et al. 1987), linear fit, negative β with shallow slope. c) *Odontesthes bonariensis* (Strussmann et al. 1997), linear fit, positive β. d) *Physignathus lesueurii* (Harlow 2004), curvilinear fit. See Appendix for details on model fitting.

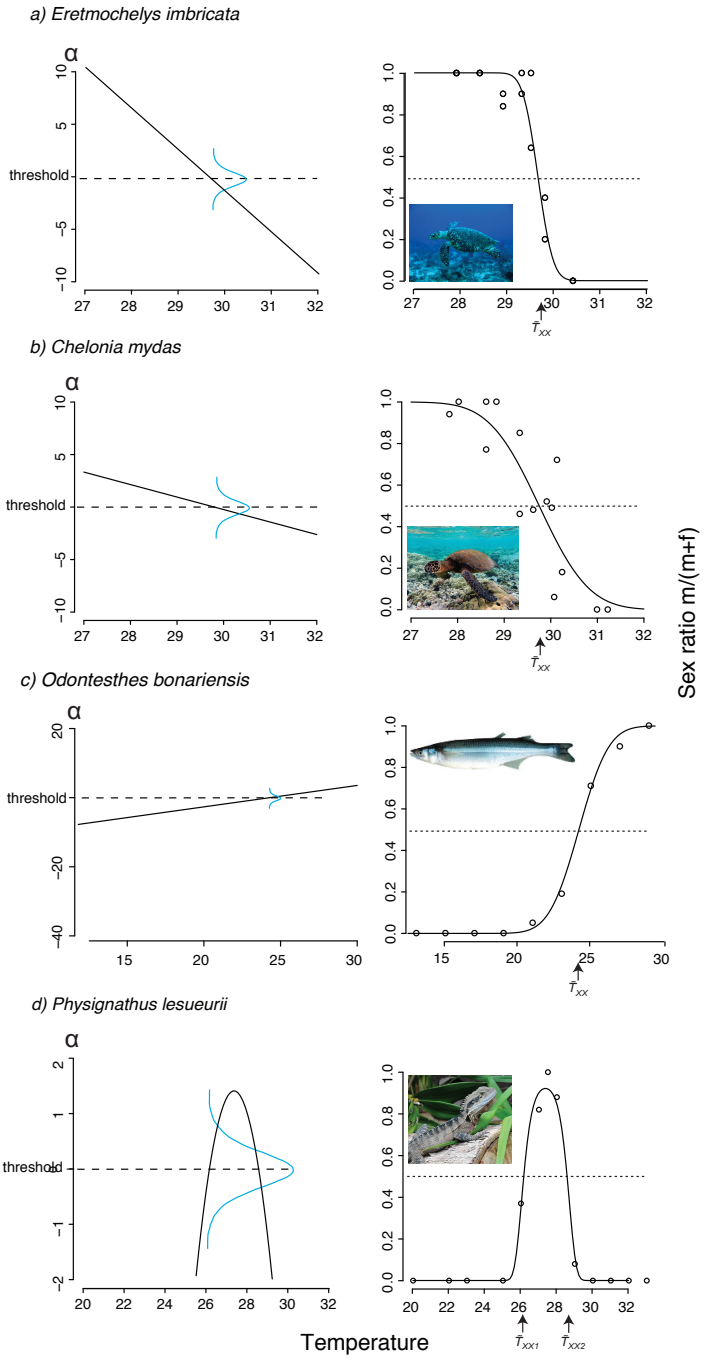


Figure 2. *Mixed systems.* Right panels: sex ratio as a function of temperature (observed points and fitted model). Pivotal temperatures are defined by horizontal dotted lines at r=0.25 and r=0.75 when two genotypes are present (a, b and d), or r=0.5 when only one is present (c, e). Left panels: corresponding reaction norms with standardized normal distribution and threshold (horizontal dashed line). a) *Poecilia reticulata* (Karayucel, Ak, & Karayucel 2006; note that sex-biased mortality cannot be ruled out) and b) *Patagonina hatcheri* (Strussmann et al. 1997) display similar interpivotal intervals but different slopes, so that changes in sex ratio are smooth and continuous in the former case, abrupt in the latter. Partial models were also fitted, with c) linear positive, d) linear negative, or e) curvilinear norms. See Appendix for details on model fitting.

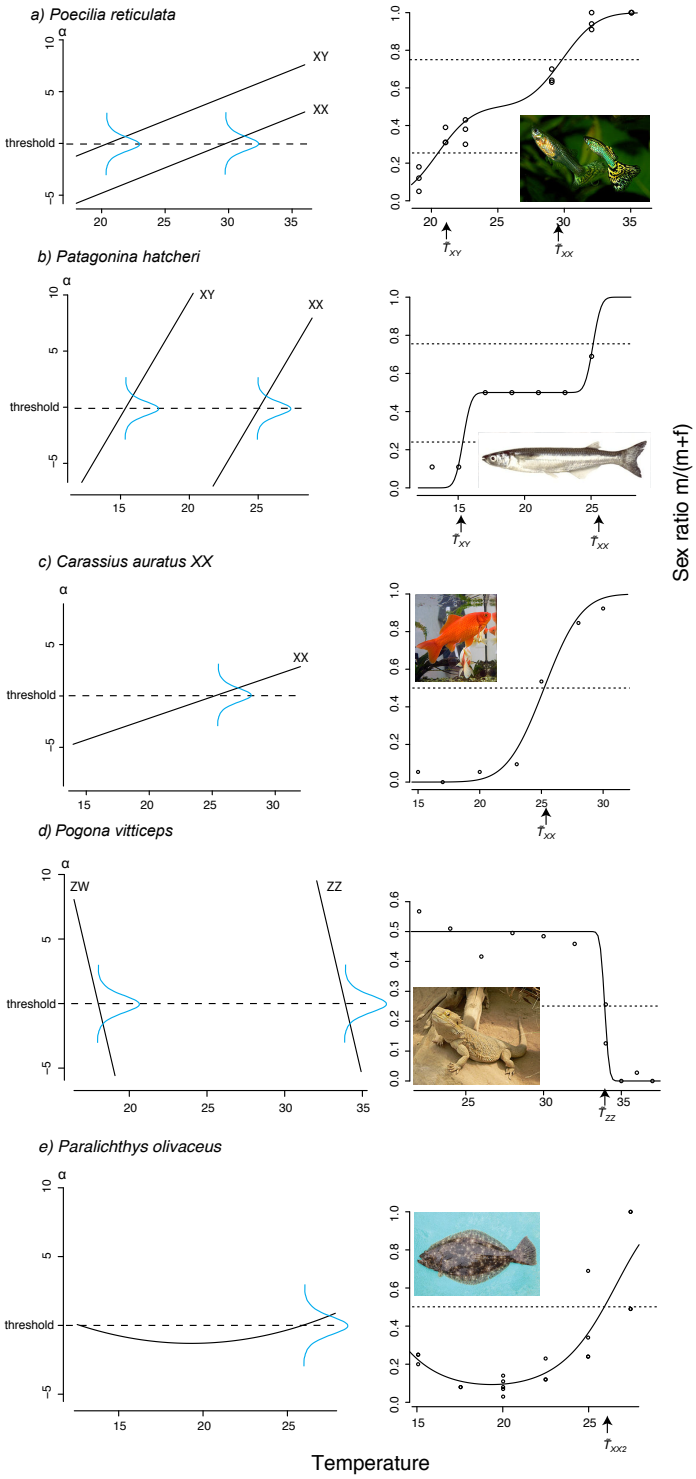
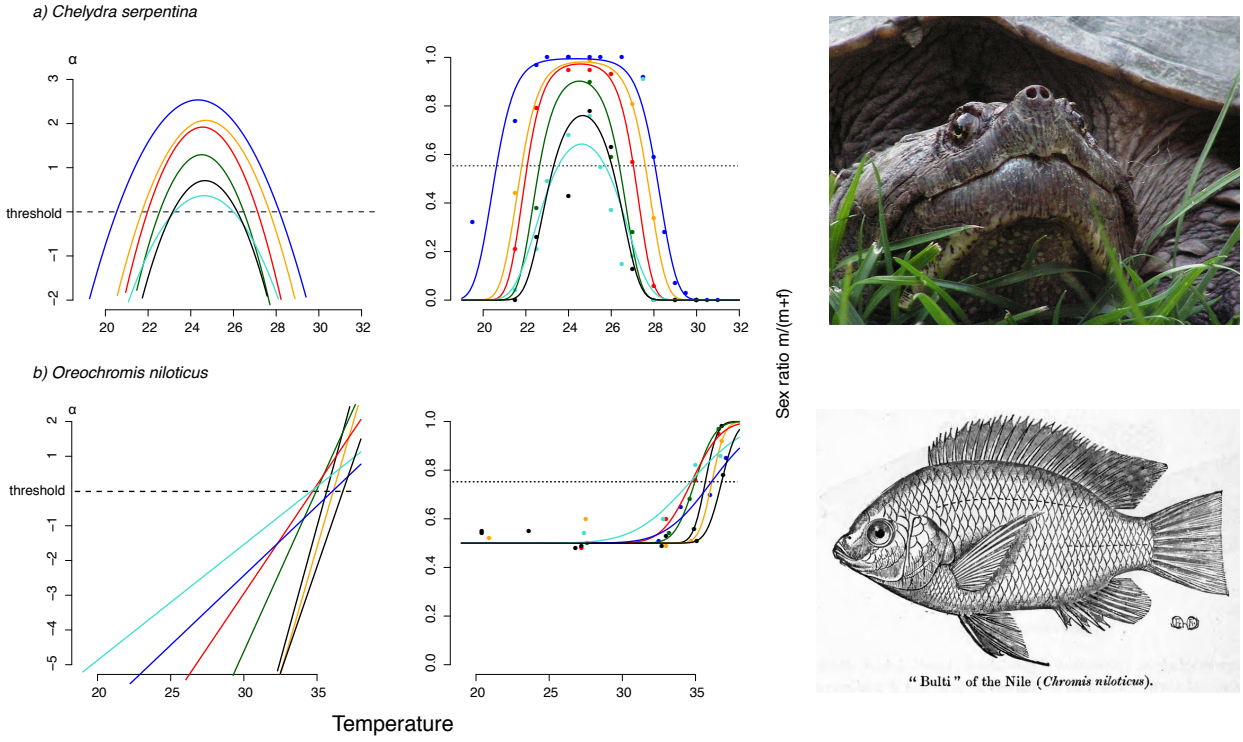


Figure 3*: Variance within / between populations.* Colors represent a) different populations of *Chelydra serpentina* (Ewert et al. 2005) with curvilinear TSD, and b) different families of *Oreochromis niloticus* (Baras et al. 2001) with linear mixed systems (partial fits). Right panels: sex ratio as a function of temperature (observed points and fitted model). Horizontal dotted lines define the pivotal temperatures (r=0.5 for TSD, r=0.75 for $\tilde{T}_{XX}$ in the mixed model). Left panels: corresponding reaction norms with threshold (horizontal dashed line). See Appendix for details on model fitting.



SUPPLEMENTARY INFORMATION

Are accessed at:

http://dl.dropbox.com/u/1035054/Chapter5_SupplementaryInformation.pdf

General Discussion and Conclusion

In the introduction of this thesis, I described the classical model of sex chromosome evolution and questioned how widely applicable this model was to broad groups of vertebrates. A question running through the entire thesis was what underlies the striking difference in sex chromosome diversity between birds and mammals (endotherms) on the one hand and amphibians, fish and reptiles (ectotherms) on the other hand. Why are sex chromosomes generally heteromorphic in the first group and mostly homomorphic in the second group? In order to tackle these questions, we first developed a general model treating sex determination as a threshold trait influenced both by genes and the environment. Second, we used this model to investigate two non-exclusive hypotheses on the striking contrast between sex determination systems in endo- versus ectothermic vertebrates. We showed, that the general temperature-dependence of physiological and therefore developmental processes of ectothermic vertebrates has the potential to increase the chances for both frequent turnovers and recombination within normally non-recombining regions of sex chromosomes.

Our formalization in **chapter one** showed that temperature changes are expected to provoke turnovers in sex determination mechanisms. Climatic changes or range expansions can, therefore, be seen as triggers for frequent turnovers in ectothermic vertebrates. Homomorphic sex chromosomes are maintained because the elapsed time would be too short to accumulate deleterious mutations or structural changes. Sex chromosomes, thereby, maintain their evolutionary potential. This study supports the turnover hypothesis and provides a broad explanation why such turnovers might be more frequent in ectothermic vertebrates.

Sexually antagonistic genes are expected to accumulate on sex chromosomes and will likely affect the dynamics of turnovers. On one hand, these genes counter-select sex reversed XY females and contribute to the stability of a sex determination system, making temperature-induced turnovers less likely. On the other hand they have the potential to favor turnovers (van Doorn & Kirkpatrick 2007; Roberts et al. 2009).

The accumulation of deleterious mutations on the Y (or W) is an important factor in sex chromosome evolution, likely influencing turnovers. O. Blaser (pers. comm.) investigates in an ongoing study whether and how the mutation load on an old sex chromosome may act as a trigger for sex chromosome turnovers, favoring a new sex determiner on an autosome.

The model developed in the first chapter of my thesis was an important foundation for the remaining chapters. Signatures of a turnover scenario as described above should be detectable in nature. In **chapter two,** we investigated a potential example of such a turnover in newts. Five subspecies of crested newts suffer from the consequences of a balanced lethal system. At each generation, the newts lose 50% of their offspring. Two

hypotheses were proposed to explain the evolution of the balanced lethal system. We proposed a third one, which stipulates that the balanced lethal system of crested newts is the result of a sex chromosome turnover. Two variants of Y-chromosomes would exist and genotypes homozygous for the Y would be lethal. We have shown that the turnover scenario may indeed be likely.

Our hypothesis might be very interesting to test empirically. We have a collection of embryos with known genotypes for chromosome pair 1 (homozygous genotypes showed an early arrest of development). Due to lowered costs and broad applicability, high-throughput sequencing became feasible for a large number of study organisms. For instance, RAD (restriction-associated DNA, Baird et al. 2008; Davey & Blaxter 2011) sequencing is applicable to non-model species, including the generation of good linkage maps and sequence data concurrently. Furthermore, the *Xenopus tropicalis* genome sequence and a large EST dataset from the tiger salamander *Ambystoma* are available online could be used as reference data. In both *X. tropicalis* and *Ambystoma,* the sex-linked linkage group is known (Smith & Voss 2009; Olmstead, Lindberg-Livingston, & Degitz 2010). The sex determiner *DM-W* has been found in *Xenopus laevis* (Yoshimoto et al. 2008) and the sex-determining factor *ambysex* in *Ambystoma* has been mapped (Smith & Voss 2009). To find signatures of old sex chromosomes on the chromosome pair 1 linkage group, I would aim to find homologous genes present on the Y of closely related species as for instance the Alpine newt *Ichthyosaura alpestris* (or if X and Y are very similar, on the sex-linked linkage group). If X and Y are differentiated enough in these closely related species, by carefully comparing relative divergence times between X and Y, a set of ancient Y genes may be identified on chromosome pair 1 of crested newts. Such a finding would strongly support the hypothesis that chromosome pair 1 was indeed a pair of ancient sex chromosomes. The chromosome pair 1 of *Triturus cristatus* might, however, be too degenerated to find clear signatures.

The turnover hypothesis alone may not account for the high proportion of homomorphic sex chromosomes in ectothermic vertebrates, because the hypothesis implied a very high rate of turnovers. In **chapter three,** we showed empirical support for the alternative hypothesis, the "fountain-of-youth". We showed that three related species of European tree frogs had the same sex determination system. The same sex-linked linkage group was found in all three species. Although recombination ceased in males prior to species divergence (i.e. > 5.4 Mya,), sequences on conspecific X and Y alleles were more similar to each other than the different Y alleles. The most likely explanation is that occasional X-Y recombination prevents the differentiation of the sex chromosomes. Sporadic sex reversals to XY females would induce XY recombination. Sex reversals occurring within habitat-specific environmental conditions were indeed documented in the closely related *H. japonica*

(Kawamura & Nishioka 1977). I am not aware of any study concerning temperature dependence of sex determination in our study species. There is, however, an ongoing collaboration in the Perrin group, which aims to produce sex-reversed tree frogs through hormonal treatments. Successfully sex-reversed individuals will be raised to reproductive maturity, with the goal to measure recombination in their offspring. The expectation is that XY females would recombine similarly to XX females. However, XX males would be expected to not recombine among sex chromosomes. Such a result would experimentally support the fountain-of-youth hypothesis.

One assumption made in chapter three was that the 5 million years since the emergence of the original sex chromosome pair would be long enough to differentiate the X and Y in absence of recombination. Furthermore, we focused only on one gene, a non-coding sequence and microsatellites. The question arises whether this relatively small sample is representative enough for all genes on the X and Y chromosome in regard to sequence divergence. In support of our study is that approximately one million years was sufficient in *Drosophila miranda* to lead to very high sequence divergence between the neo-Y and neo-X (reviewed in Charlesworth et al. 2005). Studies on sex chromosome differentiation in related tree frog species might reveal more on the generality of our findings. Alan Brelsford working in the Perrin group will investigate the divergence between tree frog X and Y using genomics and transcriptomics tools. It would be interesting to look for signatures of degeneration on the Y, by searching for X-linked genes and testing if the Y carries degenerated or functional copies. An important step forward would be to identify the sex determiner in the three studied species. However, identifying a new sex determiner has generally proven to be a very difficult task. If the *DMRT1* gene or a homolog were responsible for sex determination (as shown in *Xenopus*, Yoshimoto et al. 2008), it would support that molecular mechanisms were generally conserved in ectothermic vertebrates despite the high diversity of sex determination systems (Graves & Peichel 2010). Sex determination based on *DMRT1* would probably be dosage related and could be seen as a threshold trait.

Sexually antagonistic genes are expected to counteract sex reversals. **Chapter four** aimed to investigate how the accumulation of deleterious mutations and sexually antagonistic selection interact to influence the rate of sex reversal. By extension, the rate of sex reversal was expected to influence the recombination rate between X and Y. We showed that allelic values at the sex determiner evolved closer to the threshold if deleterious mutations were of intermediate effect. The evolution of allelic values towards the threshold increasingly led to sex-reversals. Thereby, recombination occurred between X and Y, followed by purging on the Y. Rates of sex reversal were generally low, but XY females were produced much more frequently than XX males, as only the Y

gained an advantage from sex reversals and recombination. Our model provided insight into the importance of sexually antagonistic selection in the fountain-of-youth hypothesis. Furthermore, our model showed that in species with genetic sex determination (GSD) low rates of sex-reversals might be adaptive, because they allow purging on the Y.

Sexually antagonistic selection seems to be an important trigger for sex chromosome differentiation. From this follows, that we could expect a relationship between strength of sexual dimorphism (as a rough measure of sexually antagonistic selection) and chromosome differentiation and pure GSD. A meta-analysis along these lines could give interesting insights. Instead of episodic sex-reversals, purging on the Y may also occur due to very low levels of recombination at each generation. Further modeling is required to investigate the evolutionary framework that may favor one over the other. For this, recombination rates would need to be evolvable and determined by a recombination modifier locus.

An empirical extension to chapter four may be an investigation of the genetic diversity in sexually antagonistic genes. However, it has proven difficult to identify sexually antagonistic genes in the genome. Comparison of gene expression levels between sexes (i.e. sex-biased expression analysis) might help to identify candidate genes. Linking sex-specific fitness data with genome-wide transcript abundance may also be promising (Innocenti and Morrow 2010). Mank and Ellegren (2009) combined data on regulatory changes, to identify which genes were specifically up- or down-regulated in males and females, in addition to sex-biased expression data to reduce artifacts due to incomplete dosage compensation and meiotic sex chromosome inactivation.

During my thesis, I investigated sex chromosome evolution under the assumptions that TSD and GSD are two ends of a continuum, rather than two opposing mechanisms. A continuum between TSD and GSD implies that sex ratios of GSD species might be influenced by extreme temperatures (compared to the normal habitat of the species). In **chapter five,** we reviewed different sex determining systems in vertebrates with emphasis on the influence of temperature. We discussed possible consequences of climatic changes for TSD and most notably GSD species, with the conclusion that sex-ratio biases by climatic changes may also represent an extinction threat for some GSD species. We identified GSD species with an increased risk of sex ratio biases due to their temperature susceptibility of sex determination. It would be interesting to investigate the further conditions under which the extinction risk of a TSD species may be minimized under climatic changes. For example, what is the role of generation time? A long and overlapping generation time may be beneficial as a biased sex ratio

within one cohort may be counter-balanced by other non-biased cohorts. However, long generation times slow down adaptive change for a different climate.

In chapter five, we used a very simplified model of temperature change. Future climatic changes likely include more than an increase in mean global temperature. However, climatic changes such as increased temperature fluctuations are difficult to predict. Nevertheless, the qualitative outcome of our study should not be affected; namely that ectothermic vertebrates with genetic sex determination might also be at risk.

As discussed in chapter five, inter- or intrapopulation variation of temperature dependence in sex determination was shown in several species. It would, therefore, be interesting to investigate genetic variation (within and between populations) at loci involved in temperature dependent sex determination. The common frog *Rana temporaria* has genetic sex determination with temperature susceptibility (Piquet 1930, from Wallace et al. 1999). To identify the genetic basis of sex determination in *R. temporaria*, cross-amplifications from species with a known sex determiner might be feasible as sequences of such genes are usually relatively conserved. An ongoing study in the Perrin group is focused on whether *Rana temporaria* may have among-population variation in temperature-susceptibility of sex determination along an altitudinal gradient. This study system would be suitable to identify genetic variation in sex determining genes. *CYP19*, a gene involved in the production of aromatase (an enzyme converting testosterone to estrogens) is repressed by high temperatures in tilapia (Baroiller, D'Cotta, & Saillant 2009b). *CYP19*, *DMRT1 and SOX9* were successfully amplified in *Rana rugosa*, *Xenopus tropicalis and Bufo marinus*. These three genes have been shown to be involved in sex determination or sexual development and show high sequence similarities to homologs in other vertebrates (Abramyan, Feng, & Koopman 2009). Using a nested PCR with degenerate primers, I was able to amplify a homolog of *SOX3* (known to promote *CYP19* expression) in *R. temporaria*. Further genes such as *FoxL2* and *Amh* may be additional candidates for a population genetic screen.

Classical models of sex chromosome evolution were mainly founded on empirical studies of male heterogametic systems in mammals and *Drosophila*. Despite the dissimilarity of these taxa both are characterized by highly degenerated Y-chromosomes. Advances in theoretical work in various fields were often influenced by the availability of empirical data. Knowledge on the biology of organisms is obviously influenced by model species such as some mammals and *Drosophila* species. Including poorly studied phylogenetic clades and non-model species significantly deepens the understanding of evolutionary processes acting on sex

chromosomes. However, even well studied species provide new insights into the diversity of sex determination. For example, the most likely polygenic sex determination in zebrafish (*DMRT1,* other genetic factors and environmental cues being involved, Bradley et al. 2011) and *Xenopus laevis* with homomorphic sex chromosomes (Yoshimoto et al. 2008) may be very interesting models to further investigate the interaction of various selective pressures acting on sex determination. As delineated above, *Hyla arborea* and its sister species are also promising study systems. Sex is generally determined by genotype and at least three sister species share the same homomorphic sex chromosomes despite complete recombination suppression between the sex chromosomes in males. This system would be very interesting to search for signatures of ancient sex-reversal events on the Y chromosome and to test if *XY* sex-reversed females indeed recombine as do *XX* females.

The two hypotheses investigated here, frequent turnovers (induced by climatic changes) and the fountain-of-youth are non-exclusive and both may explain at least partially the striking contrast in sex determination between endo- and ectothermic vertebrates. Due to the temperature susceptibility of sex determination, turnovers might be induced by climatic changes. Both frequent turnovers and sporadic recombination can explain the prevalence of homomorphic sex chromosomes. Homomorphic sex chromosomes are evolutionarily labile making in turn turnovers more likely. Models of sex chromosome evolution should, therefore, consider the potential for turnovers and temperature susceptibility to become more inclusive especially for ectothermic vertebrates.

The focus of my thesis was on sex determination in vertebrates, but also invertebrates show a striking diversity in sex determination, including fairly conserved systems as the *XX/XY* systems in *Drosophila* and *ZZ/ZW* in butterflies, haplodiploidy in bees, ants and wasps and environmental sex determination in certain nematodes and crustaceans (Bull 1983; Kato et al. 2011). Sex chromosomes are also observed in some plant species. The study of sex determination systems of many more non-model species, including species with presumably homomorphic sex chromosomes, will greatly expand our current understanding of the fascinating diversity in sex determination.

References

Abramyan, J., Feng, C.-W. & Koopman, P. (2009) Cloning and expression of candidate sexual development genes in the cane toad (*Bufo marinus*). *Developmental Dynamics*, **238**, 2430–2441.

Aida, T. (1921) On the inheritance of color in a fresh-water fish, *Aplocheilus latipes* Temmick and Tchlegel, with special reference to sex-linked inheritance. *Genetics*, **6**, 554–573.

Aida, T. (1936) Sex reversal in *Aplocheilus latipes* and a new explanation of sex differentiation. *Genetics*, **21**, 136–153.

Anderson, K. (1991) Chromosome evolution in Holarctic *Hyla* treefrogs.D.M. Green & S.K. Sessions (eds pp. 299–312. Academic Press, San Diego.

Andolfatto, P., Depaulis, F. & Navarro, A. (2001) Inversion polymorphisms and nucleotide variability in Drosophila. *Genetical Research*, **77**, 1–8.

Arakawa, Y., Nishida-Umehara, C., Matsuda, Y., Sutou, S. & Suzuki, H. (2002) X-chromosomal localization of mammalian Y-linked genes in two XO species of the Ryukyu spiny rat. *Cytogenetic And Genome Research*, **99**, 303–309.

Arens, P., Westende, W.V. & Bugter, R. (2000) *Microsatellite Markers from the European Tree Frog Hyla arborea, Molec*.

Arntzen, J.W., Themudo, G.E. & Wielstra, B. (2007) The phylogeny of crested newts (*Triturus cristatus* superspecies): nuclear and mitochondrial genetic characters suggest a hard polytomy, in line with the paleogeography of the centre of origin. *Contributions To Zoology*, **76**, 261–278.

Bachtrog, D. (2000) Reduced levels of microsatellite variability on the neo-Y chromosome of Drosophila miranda. *Current Biology*, **10**, 1025–1031.

Bachtrog, D. (2003a) Accumulation of Spock and Worf, two novel non-LTR retrotransposons, on the neo-Y chromosome of *Drosophila miranda. Molecular Biology And Evolution*, **20**, 173–181.

Bachtrog, D. (2003b) Adaptation shapes patterns of genome evolution on sexual and asexual chromosomes in *Drosophila*. *Nature Genetics*, **34**, 215–219.

Bagheri-Fam, S., Sinclair, A. & Koopman, P. (2010) Conserved regulatory modules in the Sox9 testis-specific enhancer predict roles for SOX, TCF/LEF, Forkhead, DMRT, and GATA proteins in vertebrate sex determination. *The International Journal of Biochemistry & Cell Biology*, **42**, 472–477.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A. & Johnson, E.A. (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE*, **3**, e3376.

Baras, E., Jacobs, B. & Melard, C. (2001) Effect of water temperature on survival, growth and phenotypic sex of mixed (XX-XY) progenies of Nile tilapia *Oreochromis niloticus*. *Aquaculture*, **192**, 187–199.

Baroiller, J.F., D'Cotta, H., Bezault, E., Wessels, S. & Hoerstgen-Schwark, G. (2009a) Tilapia sex determination: Where temperature and genetics meet. *Comparative Biochemistry And Physiology*, **153**, 30–38.

Baroiller, J.F., D'Cotta, H. & Saillant, E. (2009b) Environmental effects on fish sex determination and differentiation. *Sexual Development*, **3**, 118–135.

Baroiller, J., Guiguen, Y. & Fostier, A. (1999) Endocrine and environmental aspects of sex differentiation in fish. *Cellular And Molecular Life Sciences*, **55**, 910–931.

Basolo, A. (2001) The effect of intrasexual fitness differences on genotype frequency stability at Fisherian sex ratio equilibrium. *Annales Zoologici Fennici*, **38**, 297–304.

Bergero, R. & Charlesworth, D. (2009) The evolution of restricted recombination in sex chromosomes. *Trends In Ecology & Evolution*, **24**, 94–102.

Berlin, S. & Ellegren, H. (2004) Chicken W: A genetically uniform chromosome in a highly variable genome. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **101**, 15967–15969.

Berset-Braendli, L., Jaquiery, J., Broquet, T. & Perrin, N. (2008) Isolation and characterization of microsatellite loci for the European tree frog (*Hyla arborea*). *Molecular Ecology Resources*, **8**, 1095–1097.

Berset-Braendli, L., Jaquiery, J. & Perrin, N. (2007) Recombination is suppressed and variability reduced in a nascent Y chromosome. *Journal Of Evolutionary Biology*, **20**, 1182–1188.

Berset-Brandli, L., Jaquiery, J., Dubey, S. & Perrin, N. (2006) A sex-specific marker reveals male heterogamety in European tree frogs. *Molecular Biology And Evolution*, **23**, 1104–1106.

Berset-Brändli, L., Jaquiéry, J., Broquet, T., Ulrich, Y. & Perrin, N. (2008) Extreme heterochiasmy and nascent sex chromosomes in European tree frogs. *Proceedings Of The Royal Society B-Biological Sciences*, **275**, 1577–1585.

Bradley, K.M., Breyer, J.P., Melville, D.B., Broman, K.W., Knapik, E.W. & Smith, J.R. (2011) An snp-based linkage map for zebrafish reveals sex determination loci. *Genes Genomes Genetics*, **1**, 3–9.

Brooks, R. & Endler, J.A. (2001) Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution*, **55**, 1002–1015.

Broquet, T., Berset-Braendli, L., Emaresi, G. & Fumagalli, L. (2007) Buccal swabs allow efficient and reliable microsatellite genotyping in amphibians. *Conservation Genetics*, **8**, 509–511.

Brust, D. (1993) Maternal brood care by *Dendrobates pumilio* - a frog that feeds its young. *Journal Of Herpetology*, **27**, 96–98.

Bull, J. (1980) Sex determination in reptiles. *Quarterly Review Of Biology*, **55**, 3–21.

Bull, J. (1981) Evolution of environmental sex determination from genotypic sex determination. *Heredity*, **47**, 173–184.

Bull, J. (1983) *Evolution of sex determining mechanisms*. The Benjamin/Cummings publishing company, inc., Menlo, California.

Bull, J. (1985) Sex-ratio and nest temperature in turtles - comparing field and laboratory data. *Ecology*, **66**, 1115–1122.

Bull, J. & Charnov, E. (1977) Changes in heterogametic mechanism of sex determination. *Heredity*, **39**, 1–14.

Bulmer, M.G. & Bull, J. (1982) Models of polygenic sex determination and sex-ratio control. *Evolution*, **36**, 13–26.

Callan, H. & Lloyd, L. (1960) Lampbrush chromosomes of crested newts *Triturus cristatus* (Laurenti). *Philosophical Transactions Of The Royal Society Of London Series B-Biological Sciences*, **243**, 135–&.

Campos-Ramos, R., Harvey, S.C. & Penman, D.J. (2009) Sex-specific differences in the synaptonemal complex in the genus *Oreochromis* (Cichlidae). *Genetica*, **135**, 325–332.

Carvalho, B.A., Koerich, L.B. & Clark, A.G. (2009) Origin and evolution of Y chromosomes: *Drosophila* tales. *Trends In Genetics*, **25**, 270–277.

Caubet, Y., Hatcher, M., Mocquard, J. & Rigaud, T. (2000) Genetic conflict and changes in heterogametic mechanisms of sex determination. *Journal Of Evolutionary Biology*, **13**, 766–777.

Chandler, C.H., Phillips, P.C. & Janzen, F.J. (2009) The evolution of sex-determining mechanisms: lessons from temperature-sensitive mutations in sex determination genes in *Caenorhabditis elegans*. *Journal Of Evolutionary Biology*, **22**, 192–200.

Charlesworth, B. (1978) Model for evolution of Y chromosomes and dosage compensation. *Proceedings Of The National Academy Of Sciences Of The United States Of America* pp. 5618–5622.

Charlesworth, B. (1996) The evolution of chromosomal sex determination and dosage compensation. *Current Biology*, **6**, 149–162.

Charlesworth, B. & Charlesworth, D. (2000) The degeneration of Y chromosomes. *Philosophical Transactions Of The Royal Society Of London Series B-Biological Sciences*, **355**, 1563–1572.

Charlesworth, D., Charlesworth, B. & Marais, G. (2005) Steps in the evolution of heteromorphic sex chromosomes. *Heredity*, **95**, 118–128.

Charnier, M. (1966) Action de la temperature sur la sex-ratio chez l'embryon d'*Agama agama* (Agamidae, Lacertilien). *Comptes Rendus Des Seances De La Societe De Biologie Et De Ses Filiales*, **160**, 620–&.

Charnov, E. & Bull, J. (1977) When is sex environmentally determined. *Nature*, **266**, 829–830.

Chinnici, J. (1971) Modification of Recombination Frequency in Drosophila. I. Selection for Increased and Decreased Crossing over. *Genetics*, **69**, 71–83.

Cleland, R.E. (1972) *Oenothera: Cytogenetics and Evolution*. Academic Press, New York.

Cnaani, A., Lee, B.-Y., Zilberman, N., Ozouf-Costaz, C., Hulata, G., Ron, M., D'Hont, A., Baroiller, J.F., D'Cotta, H., Penman, D.J., Tomasino, E., Coutanceau, J.-P., Pepey, E., Shirak, A. & Kocher, T.D. (2008) Genetics of sex determination in tilapiine species. *Sexual Development*, **2**, 43–54.

Connallon, T. & Clark, A.G. (2010) Gene duplication, gene conversion and the evolution of the Y chromosome. *Genetics*, **186**, 277–U451.

Conover, D. & Heins, S. (1987) The environmental and genetic components of sex-ratio in *Menidia menidia* (Pisces: Atherinidae). *Copeia*, **3**, 732–743.

Conover, D.O. (1984) Adaptive significance of temperature-dependent sex determination in a fish. *American Naturalist*, **123**, 297–313.

Conover, D.O. & Kynard, B.E. (1981) Environmental sex determination - interaction of temperature and genotype in a fish. *Science*, **213**, 577–579.

Conover, D.O. & Vanvoorhees, D.A. (1990) Evolution of a balanced sex-ratio by frequency-dependent selection in a fish. *Science*, **250**, 1556–1558.

Conover, D., Voorhees, D. & Ehtisham, A. (1992) Sex ratio selection and the evolution of environmental sex determination in laboratory populations of *Menidia menidia*. *Evolution*, **46**, 1722–1730.

Crew, F.A.E. (1921) Sex-reversal in frogs and toads - A review of the recorded cases of abnormality of the reproductive system and an account of a breeding experiment. *Journal Of Genetics*, **11**, 141–181.

Crews, D. & Bergeron, J.M. (1994) Role of reductase and aromatase in sex determination in the Red-Eared Slider (*Trachemys-scripta*), a turtle with temperature-dependent sex determination. *Journal Of Endocrinology*, **143**, 279–289.

Crews, D., Fleming, A., Willingham, E. & Baldwin, R. (2001) Role of steroidogenic factor 1 and aromatase in temperature-dependent sex determination in the red-eared slider turtle. *Journal of Experimental Zoology*, **290**, 597–606.

D'Cotta, H., Fostier, A., Guiguen, Y., Govoroun, M. & Baroiller, J. (2001) Aromatase plays a key role during normal and temperature-induced sex differentiation of Tilapia *Oreochromis niloticus*. *Molecular Reproduction And Development*, **59**, 265–276.

Davey, J.W. & Blaxter, M.L. (2011) RADSeq: next-generation population genetics. *Briefings in Functional Genomics*, **9**, 416–423.

Dawson, P.S. (1967) A balanced lethal system in the flour beetle *Tribolium castaneum*. *Heredity*, **22**, 435–&.

De Souza, R.R. & Vogt, R.C. (1994) Incubation-temperature influences sex and hatchling size in the neotropical turtle *Podocnemis unifilis*. *Journal Of Herpetology*, **28**, 453–464.

Delmas, V., Prevot-Julliard, A.C., Pieau, C. & Girondot, M. (2008) A mechanistic model of temperature-dependent sex determination in a chelonian: the European pond turtle. *Functional Ecology*, **22**, 84–93.

Deng, H.W. & Lynch, M. (1996) Estimation of deleterious-mutation parameters in natural populations. *Genetics*, **144**, 349–360.

Desvages, G., Girondot, M. & Pieau, C. (1993) Sensitive stages for the effects of temperature on gonadal aromatase-activity in embryos of the marine turtle *Dermochelys coriacea*. *General And Comparative Endocrinology*, **92**, 54–61.

Devlin, R. & Nagahama, Y. (2002) Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*, **208**, 191–364.

Dodd, K.L., Murdock, C. & Wibbels, T. (2006) Interclutch variation in sex ratios produced at pivotal temperature in the Red-Eared Slider, a turtle with temperature-dependent sex determination. *Journal Of Herpetology*, **40**, 544–549.

Doody, J.S. (2009) Superficial lizards in cold climates: Nest site choice along an elevational gradient. *Austral Ecology*, **34**, 773–779.

Doody, J.S., Guarino, E., Georges, A., Corey, B., Murray, G. & Ewert, M. (2006) Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. *Evolutionary Ecology*, **20**, 307–330.

Dournon, C., Houillon, C. & Pieau, C. (1990) Temperature sex-reversal in amphibians and reptiles. *Int. J. Dev. Biol.*, **34**, 81–92.

Drummond, A.J., SYW, H., Rawlence, N. & Rambaut, A. A rough guide to BEAST 1.4. Available: http://code.google.com/p/beast-mcmc/downloads/detail?name= BEAST14_Manual_6July2007.pdf

Eggert, C. (2004) Sex determination: the amphibian models. *Reproduction, nutrition, development*, **44**, 539–549.

Eiby, Y.A., Wilmer, J.W. & Booth, D.T. (2008) Temperature-dependent sex-biased embryo mortality in a bird. *Proceedings Of The Royal Society B-Biological Sciences*, **275**, 2703–2706.

Ellegren, H. (2011) Sex-chromosome evolution: recent progress and the influence of male and female heterogamety. *Nature Reviews Genetics*, **12**, 157–166.

Ellegren, H. & Parsch, J. (2007) The evolution of sex-biased genes and sex-biased gene expression. *Nature Reviews Genetics*, **8**, 689–698.

Endler, J.A. (1980) Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, **34**, 76–91.

Endler, J.A. (1983) Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology Of Fishes*, **9**, 173–190.

Engelstaedter, J. (2008) Muller's ratchet and the degeneration of Y chromosomes: a simulation study. *Genetics*, **180**, 957–967.

Ewert, M.A., Echtberger, C.R. & Nelson, C.E. (2004) Turtle sex-determining modes and TSD patterns, and some TSD pattern correlates. *Temperature-dependent sex determination in vertebrates.* N. & V. Lance eds (pp. 21–32). Smithsonian Books, Washington.

Ewert, M.A., Lang, J.W. & Nelson, C.E. (2005) Geographic variation in the pattern of temperature-dependent sex determination in the American snapping turtle (*Chelydra serpentina*). *Journal of Zoology*, **265**, 81–95.

Ezaz, T., Quinn, A., Miura, I., Sarre, S., Georges, A. & Graves, J.A.M. (2005) The dragon lizard *Pogona vitticeps* has ZZ/ZW micro-sex chromosomes. *Chromosome Research*, **13**, 763–776.

Ezaz, T., Stiglec, R., Veyrunes, F. & Graves, J.A.M. (2006a) Relationships between vertebrate ZW and XY sex chromosome systems. *Current Biology*, **16**, R736–R743.

Ezaz, T., Valenzuela, N., Grutzner, F., Miura, I., Georges, A., Burke, R. & Graves, J.A.M. (2006b) An XX/XY sex microchromosome system in a freshwater turtle, *Chelodina longicollis* (Testudines : Chelidae) with genetic sex determination. *Chromosome Research*, **14**, 139–150.

Ezaz, T., Sarre, S.D., O'Meally, D., Graves, J.A.M. & Georges, A. (2009a) Sex chromosome evolution in lizards: independent origins and rapid transitions. *Cytogenetic And Genome Research*, **127**, 249–260.

Ezaz, T., Quinn, A.E., Sarre, S.D., O'Meally, D., Georges, A. & Graves, J.A.M. (2009b) Molecular marker suggests rapid changes of sex-determining mechanisms in Australian dragon lizards. *Chromosome Research*, **17**, 91–98.

Felip, A., Fujiwara, A., Young, W., Wheeler, P., Noakes, M., Phillips, R. & Thorgaard, G. (2004) Polymorphism and differentiation of rainbow trout Y chromosomes. *Genome*, **47**, 1105–1113.

Ferguson-Smith, M. (2007) The evolution of sex chromosomes and sex determination in vertebrates and the key role of DMRT1. *Sexual Development*, **1**, 2–11.

Filatov, D.A., ger, F.C.O.M.E., Negrutiu, I. & Charlesworth, D. (2000) Low variability in a Y-linked plant gene and its implications for Y-chromosome evolution. *Nature*, **404**, 388–390.

Francis, R.C. & Barlow, G.W. (1993) Social control of primary sex differentiation in the Midas cichlid. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **90**, 10673–10675.

Fraser, J.A. & Heitman, J. (2005) Chromosomal sex-determining regions in animals, plants and fungi. *Current Opinion in Genetics & Development*, **15**, 645–651.

Fredge, K. (1994) *The differences between the sexes* (RV Short and E Balaban, Eds.). Cambridge University Press, Cambridge.

Freedberg, S. & Taylor, D. (2007) Sex ratio variance and the maintenance of environmental sex determination. *Journal Of Evolutionary Biology*, **20**, 213–220.

Fridolfsson, A.K., Cheng, H., Copeland, N.G., Jenkins, N.A., Liu, H.C., Raudsepp, T., Woodage, T., Chowdhary, B., Halverson, J. &

Ellegren, H. (1998) Evolution of the avian sex chromosomes from an ancestral pair of autosomes. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **95**, 8147–8152.

Fuentes, M.M.P.B., Hamann, M. & Limpus, C.J. (2010) Past, current and future thermal profiles of green turtle nesting grounds: Implications from climate change. *Journal Of Experimental Marine Biology And Ecology*, **383**, 56–64.

Gamble, T. (2010) A review of sex determining mechanisms in geckos (Gekkota: Squamata). *Sexual Development*, **4**, 88–103.

Georges, A., Doody, S., Beggs, K. & Young, J. (2004) Thermal models of TSD under laboratory and field conditions. *Temperature-dependent sex determination in vertebrate.s* N. Valenzuela & V. Lance eds (pp. 79–89). Smithsonian Books, Washington.

Georges, A., Limpus, C. & Stoutjesdijk, R. (1994) Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. *Journal of Experimental Zoology*, **270**, 432–444.

Girondot, M. (1999) Statistical description of temperature-dependent sex determination using maximum likelihood. *Evolutionary Ecology Research*, **1**, 479–486.

Girondot, M., Ben Hassine, S., Sellos, C., Godfrey, M. & Guillon, J.M. (2010) Modeling thermal influence on animal growth and sex determination in reptiles: being closer to the target gives new views. *Sexual Development*, **4**, 29–38.

Glen, F. & Mrosovsky, N. (2004) Antigua revisited: the impact of climate change on sand and nest temperatures at a hawksbill turtle (*Eretmochelys imbricata*) nesting beach. *Global Change Biology*, **10**, 2036–2045.

Godfrey, M., D'Amato, A., Marcovaldi, M. & Mrosovsky, N. (1999) Pivotal temperature and predicted sex ratios for hatchling hawksbill turtles from Brazil. *Canadian Journal Of Zoology*, **77**, 1465–1473.

Godfrey, M., Delmas, V. & Girondot, M. (2003) Assessment of patterns of temperature-dependent sex determination using maximum likelihood model selection. *Ecoscience*, **10**, 265–272.

Gordo, I. & Campos, P. (2008) Sex and deleterious mutations. *Genetics*, **179**, 621–626.

Goth, A. & Booth, D. (2005) Temperature-dependent sex ratio in a bird. **1**, 31–33.

Goudet, J. (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal Of Heredity*, **86**, 485–486. Available: http://www2.unil.ch/popgen/softwares/fstat.htm.

Graves, J.A.M. (2006) Sex chromosome specialization and degeneration in mammals. **124**, 901–914.

Graves, J.A.M. (2008) Weird animal genomes and the evolution of vertebrate sex and sex chromosomes. *Annual Review Of Genetics*, **42**, 565–586.

Graves, J.A.M. & Peichel, C.L. (2010) Are homologies in vertebrate sex determination due to shared ancestry or to limited options? *Genome Biology*, **11**, 205.

Green, P., Falls, K.A. & Crooks, S. (Eds.). *CRIMAP* (P Green, KA Falls, and S Crooks, Eds.). St Louis, MO: Washington University School of Medicine. http://linkage.rockefeller.edu/soft/crimap/.

Grossen, C., Neuenschwander, S. & Perrin, N. (2011) Temperature-dependent turnovers in sex-determination mechanisms: a quantitative model. *Evolution*, **65**, 64–78.

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic biology*, **52**, 696–704. http://www.atgc- lirmm.fr/phyml/.

Haag, E. & Doty, A. (2005) Sex determination across evolution: Connecting the dots. *PLoS Biology*, **3**, 21–24.

Hamilton, W. (1967) Extraordinary sex ratios. *Science*, **156**, 477–&.

Harlow, P.S. (2004) Temperature-dependent sex determination in lizards. *Temperature-dependent sex determination in vertebrates.* N. Valenzuela & V. Lance eds. (pp 42-52). Smithsonian Books, Washington.

Haskins, C., Young, P., Hewitt, R. & Haskins, E. (1970) Stabilised heterozygosis of supergenes mediating certain Y-linked colour patterns in populations of *Lebistes reticulatus*. *Heredity*, **25**, 575–&.

Hattori, R.S., Gould, R.J., Fujioka, T., Saito, T., Kurita, J., Struessmann, C.A., Yokota, M. & Watanabe, S. (2007) Temperature-dependent sex determination in Hd-rR medaka *Oryzias latipes*: Gender sensitivity, thermal threshold, critical period, and DMRT1 expression profile. *Sexual Development*, **1**, 138–146.

Hawkes, L.A., Broderick, A.C., Godfrey, M.H. & Godley, B.J. (2007) Investigating the potential impacts of climate change on a marine turtle population. *Global Change Biology*, **13**, 923–932.

Hays, G., Broderick, A., Glen, F. & Godley, B. (2003) Climate change and sea turtles: a 150-year reconstruction of incubation temperatures at a major marine turtle rookery. *Global Change Biology*, **9**, 642–646.

Hedrick, P.W. (2007) Sex: Differences in mutation, recombination, selection, gene flow, and genetic drift. *Evolution*, **61**, 2750–2771.

Higgins, K. & Lynch, M. (2001) Metapopulation extinction caused by mutation accumulation. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **98**, 2928–2933.

Hillis, D. & Green, D. (1990) Evolutionary changes of heterogametic sex in the phylogenetic history of amphibians. *Journal Of Evolutionary Biology*, **3**, 49–64.

Hodgkin, J. (2002) The remarkable ubiquity of DM domain factors as regulators of sexual phenotype: ancestry or aptitude? *Genes & Development*, **16**, 2322–2326.

Hoegg, S., Vences, M., Brinkmann, H. & Meyer, A. (2004) Phylogeny and comparative substitution rates of frogs inferred from sequences of three nuclear genes. *Molecular Biology And Evolution*, **21**, 1188–1200.

Houde, A.E. (1992) Sex-linked heritability of a sexually selected character in a natural-population of *Poecilia reticulata* (Pisces, Poeciliidae) (guppies). *Heredity*, **69**, 229–235.

Hughes, J.F., Skaletsky, H., Pyntikova, T., Graves, T.A., van Daalen, S.K.M., Minx, P.J., Fulton, R.S., Mcgrath, S.D., Locke, D.P., Friedman, C., Trask, B.J., Mardis, E.R., Warren, W.C., Repping, S., Rozen, S., Wilson, R.K. & Page, D.C. (2010) Chimpanzee and human Y chromosomes are remarkably divergent in structure and gene content. *Nature*, **463**, 536–539.

Hulin, V., Delmas, V., Girondot, M., Godfrey, M.H. & Guillon, J.-M. (2009) Temperature-dependent sex determination and global change: are some species at greater risk?, **160**, 493–506.

Hurtado-Gonzales, J.L. & Uy, J.A.C. (2010) Intrasexual competition facilitates the evolution of alternative mating strategies in a colour polymorphic fish. *Bmc Evolutionary Biology*, **10**, 391.

Innocenti, P. & Morrow, E.H. (2010) The sexually antagonistic genes of Drosophila melanogaster. PLoS Biology, 8, e1000335.

Inoue, H., Fukumori, Y. & Hiroyoshi, T. (1983) Mapping of autosomal male-determining factors of the housefly, *Musca domestica* L., by means of sex-reversal. *Idengaku Zasshi*, **58**, 451–461.

IPCC Fourth Assessment Report. IPCC Fourth Assessment Report.

Janzen, F. & Krenz, J. (2004) Phylogenetics: which was first, TSD or GSD.N. Valenzuela & V.A. Lance (eds pp. 121–130. Temperature Dependent Sex Determination.

Janzen, F. & Morjan, C. (2001) Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination. *Animal Behaviour*, **62**, 73–82.

Janzen, F.J. (1992) Heritable variation for sex-ratio under environmental sex determination in the common snapping turtle (*Chelydra-serpentina*). *Genetics*, **131**, 155–161.

Janzen, F.J. (1994) Climate-change and temperature-dependent sex determination in reptiles. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **91**, 7487–7490.

Janzen, F.J. & Phillips, P.C. (2006) Exploring the evolution of environmental sex determination, especially in reptiles. *Journal Of Evolutionary Biology*, **19**, 1775–1784.

Jaquiery, J., Guillaume, F. & Perrin, N. (2009) Predicting the deleterious effects of mutation load in fragmented populations. *Conservation Biology*, **23**, 207–218.

Just, W., Rau, W., Vogel, W., Akhverdian, M., Fredga, K., Graves, J.A.M. & Lyapunova, E. (1995) Absence of Sry in species of the vole *Ellobius*. *Nature Genetics*, **11**, 117–118.

Kallimanis, A.S. (2009) Temperature dependent sex determination and climate change. *Oikos*, **119**, 197–200.

Kallman, K. (1970) Sex determination and restriction of sex-linked pigment patterns to X-chromosomes and Y-chromosomes in populations of a poeciliid fish, *Xiphophorus maculatus*, from Belize and Sibun rivers of British-Honduras. *Zoologica*, **55**, 1–16.

Karayucel, I., Ak, O. & Karayucel, S. (2006) Effect of temperature on sex ratio in guppy *Poecilia reticulata* (Peters 1860). *Aquaculture Research*, **37**, 139–150.

Kato, Y., Kobayashi, K., Watanabe, H. & Iguchi, T. (2011) Environmental sex determination in the branchiopod crustacean *Daphnia magna*: deep conservation of a doublesex gene in the sex-determining pathway (A Kopp, Ed.). *PLoS Genetics*, **7**, e1001345.

Kawai, A., Nishida-Umehara, C., Ishijima, J., Tsuda, Y., Ota, H. & Matsuda, Y. (2007) Different origins of bird and reptile sex chromosomes inferred from comparative mapping of chicken Z-linked genes. *Cytogenetic And Genome Research*, **117**, 92–102.

Kawamura, T. & Nishioka, M. (1977) *The reproductive biology of amphibians* (D Taylor and S Guttman, Eds.). Plenum Publishing Corporation, New York & London.

Kettlewell, J., Raymond, C. & Zarkower, D. (2000) Temperature-dependent expression of turtle Dmrt1 prior to sexual differentiation. *Genesis*, **26**, 174–178.

Kimura, M. (1983) *The neutral theory of molecular evolution*. Cambridge University Press.

Kingston, J., Rosenthal, G. & Ryan, M. (2003) The role of sexual selection in maintaining a colour polymorphism in the pygmy swordtail, *Xiphophorus pygmaeus*. *Animal Behaviour*, **65**, 735–743.

Kirkpatrick, M., Guerrero, R.F. & Scarpino, S.V. (2010) Patterns of neutral genetic variation on recombining sex chromosomes. *Genetics*, **184**, 1141–1152.

Kobayashi, A. (2005) Sox9 in Testis Determination. *Annals of the New York Academy of Sciences*, **1061**, 9–17.

Kondo, M., Nagao, E., Mitani, H. & Shima, A. (2001) Differences in recombination frequencies during female and male meioses of the sex chromosomes of the medaka, *Oryzias latipes*. *Genetical Research*, **78**, 23–30.

Kondo, M., Nanda, I., Hornung, U., Schmid, M. & Schartl, M. (2004) Evolutionary origin of the medaka Y chromosome. *Current Biology*, **14**, 1664–1669.

Kozielska, M., Pen, I., Beukeboom, L. & Weissing, F. (2006) Sex ratio selection and multi-factorial sex determination in the housefly: a dynamic model. *Journal Of Evolutionary Biology*, **19**, 879–888.

Kraak, S. & Pen, I. (2002) Sex determining mechanisms in vertebrates. *Sex ratios: concepts and research methods* I. Hardy (ed pp. 158–177. Cambridge University Press, Cambridge, UK.

Kuroda-Kawaguchi, T., Skaletsky, H., Brown, L., Minx, P., Cordum, H., Waterston, R., Wilson, R., Silber, S., Oates, R., Rozen, S. & Page, D. (2001) The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nature Genetics*, **29**, 279–286.

Lagomorsino, I.V. & Conover, D.O. (1993) Variation in environmental and genotypic sex-determining mechanisms across a latitudinal gradient in the fish, *Menidia menidia*. *Evolution*, **47**, 487–494.

Lahn, B. & Page, D. (1999) Four evolutionary strata on the human X chromosome. *Science*, **286**, 964–967.

Lance, V.A. (2009) Is regulation of aromatase expression in reptiles the key to understanding temperature-dependent sex determination? *Journal of Experimental Zoology*, **311A**, 314–322.

Lawson-Handley, L., Ceplitis, H. & Ellegren, H. (2004) Evolutionary strata on the chicken Z chromosome: Implications for sex chromosome evolution. *Genetics*, **167**, 367–376.

Lindholm, A. & Breden, F. (2002) Sex chromosomes and sexual selection in poeciliid fishes. *American Naturalist*, **160 Suppl 6**, S214–24.

Lindholm, A.K., Brooks, R. & Breden, F. (2004) Extreme polymorphism in a Y-linked sexually selected trait. *Heredity*, **92**, 156–162.

Lyapunova, E. & Vorontsov, N. (1975) Zygotic mortality in *Ellobius lutescens* (Rodentia: Microtinae). *Experientia*, **31**, 417–418.

Lynch, M., Blanchard, J., Houle, D., Kibota, T., Schultz, S., Vassilieva, L. & Willis, J. (1999) Perspective: spontaneous deleterious mutation. *Evolution*, **53**, 645–663.

Lynn, A., Schrump, S., Cherry, J., Hassold, T. & Hunt, P. (2005) Sex, not genotype, determines recombination levels in mice. *American Journal Of Human Genetics*, **77**, 670–675.

Macgregor, H.C. & Horner, H. (1980) Heteromorphism for chromosome 1, a requirement for normal development in crested newts. *Chromosoma*, **76**, 111–122.

Maddock, M. & Schwartz, F. (1996) Elasmobranch cytogenetics: Methods and sex chromosomes. *Bulletin Of Marine Science*, **58**, 147–155.

Magerhans, A. & Hoerstgen-Schwark, G. (2010) Selection experiments to alter the sex ratio in rainbow trout (*Oncorhynchus mykiss*) by means of temperature treatment. *Aquaculture*, **306**, 63–67.

Mank, J.E. & Avise, J.C. (2009) Evolutionary diversity and turn-over of sex determination in teleost fishes. *Sexual Development*, **3**, 60–67.

Mank, J.E. & Ellegren, H. (2009) Sex-linkage of sexually antagonistic genes is predicted by female, but not male, effects in birds. *Evolution*, **63**, 1464–1472.

Mank, J., Promislow, D. & Avise, J. (2006) Evolution of alternative sex-determining mechanisms in teleost fishes. *Biological Journal Of The Linnean Society*, **87**, 83–93.

Marais, G. & Galtier, N. (2003) Sex chromosomes: how X-Y recombination stops. *Current Biology*, **13**, R641–R643.

Marais, G.A.B., Campos, P.R.A. & Gordo, I. (2010) Can Intra-Y Gene Conversion Oppose the Degeneration of the Human Y Chromosome?: A Simulation Study. *Genome Biology and Evolution*, **2**, 347–357.

Martinez, P.A., Ezaz, T., Valenzuela, N., Georges, A. & Graves, J.A.M. (2008) An XX/XY heteromorphic sex chromosome system in the Australian chelid turtle *Emydura macquarii*: A new piece in the puzzle of sex chromosome evolution in turtles. *Chromosome Research*, **16**, 815–825.

Matsuba, C., Alho, J.S. & Merila, J. (2010) Recombination rate between sex chromosomes depends on phenotypic sex in the common frog. *Evolution*, **64**, 3634–3637.

Matsuba, C., Miura, I. & Merila, J. (2008) Disentangling genetic vs. environmental causes of sex determination in the common frog, *Rana temporaria*. *BMC Genetics*, **9**, 3.

Matsuda, M., Sotoyama, S., Hamaguchi, S. & Sakaizumi, M. (1999) Male-specific restriction of recombination frequency in the sex chromosomes of the medaka, *Oryzias latipes*. *Genetical Research*, **73**, 225–231.

Matsuda, M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., Morrey, C., Shibata, N., Asakawa, S., Shimizu, N., Hori, H., Hamaguchi, S. & Sakaizumi, M. (2002) DMY is a Y-specific DM-domain gene required for male development in the medaka fish. *Nature*, **417**, 559–563.

McGaugh, S.E., Schwanz, L.E., Bowden, R.M., Gonzalez, J.E. & Janzen, F.J. (2010) Inheritance of nesting behaviour across natural environmental variation in a turtle with temperature-dependent sex determination. *Proceedings Of The Royal Society B-Biological Sciences*, **277**, 1219–1226.

Mitchell, N. & Janzen, F.J. (2010) Temperature-dependent sex determination and contemporary climate change. *Sexual Development*, **4**, 129–140.

Mitchell, N.J., Kearney, M.R., Nelson, N.J. & Porter, W.P. (2008) Predicting the fate of a living fossil: how will global warming affect sex determination and hatching phenology in tuatara? *Proceedings Of The Royal Society B-Biological Sciences*, **275**, 2185–2193.

Mitchell, N.J., Allendorf, F.W., Keall, S.N., Daugherty, C.H. & Nelson, N.J. (2010) Demographic effects of temperature-dependent sex determination: will tuatara survive global warming? *Global Change Biology*, **16**, 60–72.

Miura, I. (2008) An evolutionary witness: the frog *Rana rugosa* underwent change of heterogametic sex from XY male to ZW female. *Sexual Development*, **1**, 323–331.

Miura, I., Ohtani, H., Nakamura, M., Ichikawa, Y. & Saitoh, K. (1998) The origin and differentiation of the heteromorphic sex chromosomes Z, W, X, and Y in the frog *Rana rugosa*, inferred from the sequences of a sex-linked gene, ADP/ATP translocase. *Molecular Biology And Evolution*, **15**, 1612–1619.

Morgan, G.T. (1978) Absence of chiasmata from the heteromorphic region of chromosome I during spermatogenesis in *Triturus cristatus carnifex*. *Chromosoma*, **66**, 269–280.

Morjan, C. (2003) How rapidly can maternal behavior affecting primary sex ratio evolve in a reptile with environmental sex determination? *American Naturalist*, **162**, 205–219.

Morton, N. (1956) The detection and estimation of linkage between the genes for elliptocytosis and the rh-blood type. *American Journal Of Human Genetics*, **8**, 80–96.

Mrosovsky, N. & Provancha, J. (2009) Sex ratio of hatchling loggerhead sea turtles: data and estimates from a 5-year study. *Canadian Journal of Zoology*, 530–538.

Mulcahy, D.G. & Mendelson, J.R. (2000) Phylogeography and speciation of the morphologically variable, widespread species *Bufo valliceps*, based on molecular evidence from mtDNA. *Molecular phylogenetics and evolution*, **17**, 173–189.

Nagler, J., Bouma, J., Thorgaard, G. & Dauble, D. (2001) High incidence of a male-specific genetic marker in phenotypic female Chinook salmon from the Columbia River. *Environmental Health Perspectives*, **109**, 67–69.

Nakamura, M. (2009) Sex determination in amphibians. *Seminars In Cell & Developmental Biology*, **20**, 271–282.

Nakamura, M. (2010) The mechanism of sex determination in vertebrates-are sex steroids the key-factor? *Journal of Experimental Zoology*, **313A**, 381–398.

Nanda, I., Kondo, M., Hornung, U., Asakawa, S., Winkler, C., Shimizu, A., Shan, Z., Haaf, T., Shimizu, N., Shima, A., Schmid, M. & Schartl, M. (2002) A duplicated copy of *DMRT1* in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes*. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **99**, 11778–11783.

Neuenschwander, S., Hospital, F., Guillaume, F. & Goudet, J. (2008) quantiNemo: an individual-based program to simulate quantitative traits with explicit genetic architecture in a dynamic metapopulation. *Bioinformatics*, **24**, 1552–1553.

Nicolas, M., Marais, G., Hykelova, V., Janousek, B., Laporte, V., Vyskot, B., Mouchiroud, D., Negrutiu, I., Charlesworth, D. & Monéger, F. (2005) A gradual process of recombination restriction in the evolutionary history of the sex chromosomes in dioecious plants. *PLoS Biology*, **3**, e4.

Niculita-Hirzel, H., Stöck, M. & Perrin, N. (2008) A key transcription cofactor on the nascent sex chromosomes of European tree frogs (*Hyla arborea*). *Genetics*, **179**, 1721–1723.

Ogielska, M. (2009) *Reproduction of amphibians*. Science Publishers, Enfield.

Ohno, S. (1967) *Sex chromosomes and sex linked genes*. Springer-Verlag, New York.

Olmo, E. (1986) Reptilia. *Animal Cytogenetics*J. Bernard (ed pp. 1–101. Gebrüder Borntraeger, Berlin.

Olmstead, A.W., Lindberg-Livingston, A. & Degitz, S.J. (2010) Genotyping sex in the amphibian, *Xenopus* (Silurana) *tropicalis*, for endocrine disruptor bioassays. *Aquatic Toxicology*, **98**, 60–66.

Organ, C.L., Janes, D.E., Meade, A. & Pagel, M. (2009) Genotypic sex determination enabled adaptive radiations of extinct marine reptiles. *Nature*, **461**, 389–392.

Orzack, S., Sohn, J., Kallman, K., Levin, S. & Johnston, R. (1980) Maintenance of the 3 sex-chromosome polymorphism in the platyfish, *Xiphophorus maculatus*. *Evolution*, **34**, 663–672.

Ospina-Álvarez, N. & Piferrer, F. (2008) Temperature-dependent sex determination in fish revisited: prevalence, a single sex ratio response pattern, and possible effects of climate change. *PLoS ONE*, **3**, e2837.

Ounap, K., Uibo, O., Zordania, R., Kiho, L., Ilus, T., Oiglane-Shlik, E. & Bartsch, O. (2004) Three patients with 9p deletions including *DMRT1* and *DMRT2*: A girl with XY complement, bilateral ovotestes, and extreme growth retardation, and two XX females with normal pubertal development. *American Journal Of Medical Genetics Part A*, **130A**, 415–423.

Payer, B. & Lee, J.T. (2008) X chromosome dosage compensation: how mammals keep the balance. *Annual Review Of Genetics*, **42**, 733–772.

Pàldi, A., Gyapay, G. & Jami, J. (1995) Imprinted chromosomal regions of the human genome display sex-specific meiotic recombination frequencies. *Current Biology*, **5**, 1030–1035.

Peichel, C.L., Ross, J.A., Matson, C.K., Dickson, M., Grimwood, J., Schmutz, J., Myers, R.M., Mori, S., Schluter, D. & Kingsley, D.M. (2004) The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. *Current Biology*, **14**, 1416–1424.

Pen, I., Uller, T., Feldmeyer, B., Harts, A., While, G.M. & Wapstra, E. (2010) Climate-driven population divergence in sex-determining systems. *Nature*, **468**, 436–438.

Perrin, N. (2009) Sex reversal: a fountain of youth for sex chromosomes? *Evolution*, **63**, 3043–3049.

Phadnis, N. & Fry, J.D. (2005) Widespread correlations between dominance and homozygous effects of mutations: implications for theories of dominance. *Genetics*, **171**, 385–392.

Pokorna, M. & Kratochvil, L. (2009) Phylogeny of sex-determining mechanisms in squamate reptiles: are sex chromosomes an evolutionary trap? *Zoological Journal Of The Linnean Society*, **156**, 168–183.

Potrzebowski, L., Vinckenbosch, N., Marques, A.C., Chalmel, F., Jegou, B. & Kaessmann, H. (2008) Chromosomal gene movements reflect the recent origin and biology of therian sex chromosomes. *PLoS Biology*, **6**, 709–716.

Quinn, A.E., Georges, A., Sarre, S.D., Guarino, F., Ezaz, T. & Graves, J.A.M. (2007) Temperature sex reversal implies sex gene dosage in a reptile. *Science*, **316**, 411.

R Development Core Team. R: A Language and Environment for Statistical Computing.

Radder, R.S., Quinn, A.E., Georges, A., Sarre, S.D. & Shine, R. (2008) Genetic evidence for co-occurrence of chromosomal and thermal sex-determining systems in a lizard. **4**, 176–178.

Ramsey, M. & Crews, D. (2009) Steroid signaling and temperature-dependent sex determination - Reviewing the evidence for early action of estrogen during ovarian determination in turtles. *Seminars In Cell & Developmental Biology*, **20**, 283–292.

Rankin, D.J. & Kokko, H. (2007) Do males matter? The role of males in population dynamics. *Oikos*, **116**, 335–348.

Raymond, C., Shamu, C., Shen, M., Seifert, K., Hirsch, B., Hodgkin, J. & Zarkower, D. (1998) Evidence for evolutionary conservation of sex-determining genes. *Nature*, **391**, 691–695.

Raymond, C., Murphy, M., O'Sullivan, M., Bardwell, V. & Zarkower, D. (2000) Dmrt1, a gene related to worm and fly sexual regulators, is required for mammalian testis differentiation. *Genes & Development*, **14**, 2587–2595.

Raymond, C.S., Parker, E.D., Kettlewell, J.R., Brown, L.G., Page, D.C., Kusz, K., Jaruzelska, J., Reinberg, Y., Flejter, W.L., Bardwell, V.J., Hirsch, B. & Zarkower, D. (1999) A region of human chromosome 9p required for testis development contains two genes related to known sexual regulators. *Human molecular genetics*, **8**, 989–996.

Repping, S., van Daalen, S.K.M., Korver, C.M., Brown, L.G., Marszalek, J.D., Gianotten, J., Oates, R.D., Silber, S., van der Veen, F., Page, D.C. & Rozen, S. (2004) A family of human Y chromosomes has dispersed throughout northern Eurasia despite a 1.8-Mb deletion in the azoospermia factor c region. *Genomics*, **83**, 1046–1052.

Rhen, T. & Lang, J. (1998) Among-family variation for environmental sex determination in reptiles. *Evolution*, **52**, 1514–1520.

Rice, W. (1984) Sex chromosomes and the evolution of sexual dimorphism. *Evolution*, **38**, 735–742.

Rice, W. (1987) The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex-chromosomes. *Evolution*, **41**, 911–914.

Rice, W. (1992) Sexually antagonistic genes: experimental evidence. *Science*, **256**, 1436–1439.

Rice, W. (1996) Evolution of the Y sex chromosome in animals. *Bioscience*, **46**, 331–343.

Robert, K. & Thompson, M. (2001) Sex determination - viviparous lizard selects sex of embryos. *Nature*, **412**, 698–699.

Roberts, R.B., Ser, J.R. & Kocher, T.D. (2009) Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi cichlid fishes. *Science*, **326**, 998–1001.

Roff, D. (1996) The evolution of threshold traits in animals. *Quarterly Review Of Biology*, **71**, 3–35.

Romer, U. & Beisenherz, W. (1996) Environmental determination of sex in *Apistogramma* (Cichlidae) and two other freshwater fishes (Teleostei). *Journal Of Fish Biology*, **48**, 714–725.

Ross, J.A., Urton, J.R., Boland, J., Shapiro, M.D. & Peichel, C.L. (2009) Turnover of sex chromosomes in the stickleback fishes (gasterosteidae). *PLoS Genetics*, **5**, e1000391.

Rosser, Z. & Balaresque, P. (2009) Gene conversion between the X chromosome and the male-specific region of the Y chromosome at a translocation hotspot. *The American Journal of Human Genetics*.

Rowe, G., Harris, D. & Beebee, T. (2006) Lusitania revisited: A phylogeographic analysis of the natterjack toad *Bufo calamita* across its entire biogeographical range. *Molecular phylogenetics and evolution*, **39**, 335–346.

Rozen, S., Skaletsky, H., Marszalek, J., Minx, P., Cordum, H., Waterston, R., Wilson, R. & Page, D. (2003) Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. *Nature*, **423**, 873–876.

Rozen, S., Marszalek, J. & Alagappan, R. (2009) Remarkably little variation in proteins encoded by the Y chromosome's single-copy genes, implying effective purifying selection. *The American Journal of Human Genetics*, **85**, 923–928.

Sarre, S.D., Georges, A. & Quinn, A. (2004) The ends of a continuum: genetic and temperature-dependent sex determination in reptiles. *BioEssays*, **26**, 639–645.

Sato, T., Endo, T., Yamahira, K., Hamaguchi, S. & Sakaizumi, M. (2005) Induction of female-to-male sex reversal by high temperature treatment in medaka, *Oryzias latipes*. *Zoological Science*, **22**, 985–988.

Schartl, M. (2004) Sex chromosome evolution in non-mammalian vertebrates. *Current Opinion in Genetics & Development*, **14**, 634–641.

Schwanz, L.E. & Janzen, F.J. (2008) Climate change and temperature-dependent sex determination: can individual plasticity in nesting phenology prevent extreme sex ratios? *Physiological and Biochemical Zoology*, **81**, 826–834.

Schwanz, L.E., Janzen, F.J. & Proulx, S.R. (2010) Sex allocation based on relative and absolute condition. *Evolution*, **64**, 1331–1345.

Scotti, I. & Delph, L.F. (2006) Selective trade-offs and sex-chromosome evolution in *Silene latifolia*. *Evolution*, **60**, 1793–1800.

Sessions, S., Macgregor, H., Schmid, M. & Haaf, T. (1988) Cytology, embryology, and evolution of the developmental arrest syndrome in newts of the genus *Triturus* (Caudata, Salamandridae). *Journal of Experimental Zoology*, **248**, 321–334.

Shine, R., Elphick, M. & Donnellan, S. (2002) Co-occurrence of multiple, supposedly incompatible modes of sex determination in a lizard population. *Ecology Letters*, **5**, 486–489.

Shoemaker, C., Ramsey, M., Queen, J. & Crews, D. (2007) Expression of Sox9, Mis, and Dmrt1 in the gonad of a species with temperature-dependent sex determination. *Developmental Dynamics*, **236**, 1055–1063.

Sims, S.H., Macgregor, H.C., Pellatt, P.S. & Horner, H.A. (1984) Chromosome-1 in crested and marbled newts (*Triturus*) - an extraordinary case of heteromorphism and independent chromosome evolution. *Chromosoma*, **89**, 169–185.

Skaletsky, H., Kuroda-Kawaguchi, T., Minx, P., Cordum, H., Hillier, L., Brown, L., Repping, S., Pyntikova, T., Ali, J., Bieri, T., Chinwalla, A., Delehaunty, A., Delehaunty, K., Du, H., Fewell, G., Fulton, L., Fulton, R., Graves, T., Hou, S., Latrielle, P., Leonard, S., Mardis, E., Maupin, R., McPherson, J., Miner, T., Nash, W., Nguyen, C., Ozersky, P., Pepin, K., Rock, S., Rohlfing, T., Scott, K., Schultz, B., Strong, C., Tin-Wollam, A., Yang, S., Waterston, R., Wilson, R., Rozen, S. & Page, D. (2003) The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature*, **423**, 825–U2.

Slattery, J.P., Sanner-Wachter, L. & O'Brien, S.J. (2000) Novel gene conversion between XY homologues located in the nonrecombining region of the Y chromosome in Felidae (Mammalia). *Proceedings Of The National Academy Of Sciences Of The United States Of America* pp. 5307–5312.

Smalley, S.L. (1993) Sex-specific recombination frequencies: a consequence of imprinting? *American Journal Of Human Genetics*, **52**, 210–212.

Smith, C.A., Roeszler, K.N., Ohnesorg, T., Cummins, D.M., Farlie, P.G., Doran, T.J. & Sinclair, A.H. (2009) The avian Z-linked gene DMRT1 is required for male sex determination in the chicken. *Nature*, **461**, 267–271.

Smith, J.J. & Voss, S.R. (2009) Amphibian sex determination: segregation and linkage analysis using members of the tiger salamander species complex (*Ambystoma mexicanum* and *A. t. tigrinum*). **102**, 542–548.

Spotila, J.R., Standora, E.A., Morreale, S.J. & Ruiz, G.J. (1987) Temperature-dependent sex determination in the green turtle (*Chelonia mydas*) - effects on the sex-ratio on a natural nesting beach. *Herpetologica*, **43**, 74–81.

Steinemann, M. & Steinemann, S. (1998) Enigma of Y chromosome degeneration: Neo-Y and Neo-X chromosomes of *Drosophila miranda* a model for sex chromosome evolution. *Genetica*, **102-3**, 409–420.

Steinemann, S. & Steinemann, M. (2005) Y chromosomes: born to be destroyed. *BioEssays*, **27**, 1076–1083.

Steinfartz, S., Vicario, S., Arntzen, J.W. & Caccone, A. (2007) A Bayesian approach on molecules and behavior: Reconsidering phylogenetic and evolutionary patterns of the Salamandridae with emphasis on Triturus newts. *Journal Of Experimental Zoology*, **308B**, 139–162.

Stöck, M., Dubey, S., Klütsch, C., Litvinchuk, S.N., Scheidt, U. & Perrin, N. (2008) Mitochondrial and nuclear phylogeny of circum-Mediterranean tree frogs from the *Hyla arborea* group. *Molecular phylogenetics and evolution*, **49**, 1019–1024.

Stöck, M., Croll, D., Dumas, Z., Biollay, S., Wang, J. & Perrin, N. (2011a) A cryptic heterogametic transition revealed by sex-linked DNA markers in Palearctic green toads. *Journal Of Evolutionary Biology*, **24**, 1064–1070.

Stöck, M., Horn, A., Grossen, C., Lindtke, D., Sermier, R., Betto-Colliard, C., Dufresnes, C., Bonjour, E., Dumas, Z., Luquet, E., Maddalena, T., Sousa, H.C., Martinez-Solano, I. & Perrin, N. (2011b) Ever-young sex chromosomes in European tree frogs. *PLoS Biology*, **9**, e1001062.

Strussmann, C., Cota, J., Phonlor, G., Higuchi, H. & Takashima, F. (1996) Temperature effects on sex differentiation of two south American atherinids, *Odontesthes argentinensis* and *Patagonina hatcheri*. *Environmental Biology Of Fishes*, **47**, 143–154.

Strussmann, C., Saito, T., Usui, M., Yamada, H. & Takashima, F. (1997) Thermal thresholds and critical period of thermolabile sex determination in two altherinid fishes, *Odontesthes bonariensis* and *Patagonina hatcheri*. *Journal of Experimental Zoology*, **278**, 167–177.

Sullivan, J.A. & Schultz, R.J. (1986) Genetic and environmental basis of variable sex-ratios in laboratory strains of *Poeciliopsis lucida*. *Evolution*, **40**, 152–158.

Sutou, S., Mitsui, Y. & Tsuchiya, K. (2001) Sex determination without the Y Chromosome in two Japanese rodents Tokudaia osimensis osimensis and Tokudaia osimensis spp. *Mammalian Genome*, **12**, 17–21.

Tanaka, K., Takehana, Y., Naruse, K., Hamaguchi, S. & Sakaizumi, M. (2007) Evidence for different origins of sex chromosomes in closely related *Oryzia* fishes: substitution of the master sex-determining gene. *Genetics*, **177**, 2075–2081.

Tease, C. & Hultén, M.A. (2004) Inter-sex variation in synaptonemal complex lengths largely determine the different recombination rates in male and female germ cells. *Cytogenetic And Genome Research*, **107**, 208–215.

Telemeco, R.S., Elphick, M.J. & Shine, R. (2009) Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. *Ecology*, **90**, 17–22.

Thompson, M., Packard, G., Packard, M. & Rose, B. (1996) Analysis of the nest environment of tuatara *Sphenodon punctatus*. *Journal of Zoology*, **238**, 239–251.

Traut, W. & Willhoeft, U. (1990) A jumping sex determining factor in the fly *Megaselia scalaris*. *Chromosoma*, **99**, 407–412.

Tripathi, N., Hoffmann, M., Weigel, D. & Dreyer, C. (2009) Linkage analysis reveals the independent origin of Poeciliid sex chromosomes and a case of atypical sex inheritance in the guppy (*Poecilia reticulata*). *Genetics*, **182**, 365–374.

Trombetta, B., Cruciani, F., Underhill, P.A., Sellitto, D. & Scozzari, R. (2010) Footprints of X-to-Y Gene Conversion in Recent Human Evolution. *Molecular Biology And Evolution*, **27**, 714–725.

Valenzuela, N. & Lance, V. (2004) *Temperature-dependent sex determination in vertebrates* (N Valenzuela and V Lance, Eds.). Smithsonian Books, Washington.

Valenzuela, N., Adams, D. & Janzen, F.J. (2003) Pattern does not equal process: Exactly when is sex environmentally determined? *American Naturalist*, **161**, 676–683.

Valenzuela, N., Botero, R. & Martinez, E. (1997) Field study of sex determination in *Podocnemis expansa* from Colombian amazonia. *Herpetologica*, **53**, 390–398.

van Dooren, T. & Leimar, O. (2003) The evolution of environmental and genetic sex determination in fluctuating environments. *Evolution*, **57**, 2667–2677.

van Doorn, G.S. & Kirkpatrick, M. (2007) Turnover of sex chromosomes induced by sexual conflict. *Nature*, **449**, 909–912.

Veyrunes, F., Waters, P.D., Miethke, P., Rens, W., McMillan, D., Alsop, A.E., Gruetzner, F., Deakin, J.E., Whittington, C.M., Schatzkamer, K., Kremitzki, C.L., Graves, T., Ferguson-Smith, M.A., Warren, W. & Graves, J.A.M. (2008) Bird-like sex chromosomes of platypus imply recent origin of mammal sex chromosomes. *Genome Research*, **18**, 965–973.

Viets, B., Tousignant, A., Ewert, M.A., Nelson, C.E. & Crews, D. (1993) Temperature-dependent sex determination in the leopard gecko, *Eublepharis macularius*. *Journal of Experimental Zoology*, **265**, 679–683.

Viets, B., Ewert, M.A., Talent, L.G. & Nelson, C.E. (1994) Sex-determining mechanisms in squamate reptiles. *Journal of Experimental Zoology*.

Volff, J.N., Nanda, I., Schmid, M. & Schartl, M. (2007) Governing sex determination in fish: Regulatory putsches and ephemeral dictators. *Sexual Development*, **1**, 85–99.

Wallace, H. (1984) Chromosome 1 and sex determination of crested newts. *Caryologia*, **37**, 3–8.

Wallace, H. (1987) Abortive development in the crested newt *Triturus cristatus*. *Development*, **100**, 65–72.

Wallace, H. & Wallace, B. (2000) Sex reversal of the newt *Triturus cristatus* reared at extreme temperatures. *International Journal of Developmental Biology*, **44**, 807–810.

Wallace, H., Badawy, G. & Wallace, B. (1999) Amphibian sex determination and sex reversal. *Cellular And Molecular Life Sciences*, **55**, 901–909.

Wallace, H., Wallace, B. & Badawy, G. (1997) Lampbrush chromosomes and chiasmata of sex-reversed crested newts. *Chromosoma*, **106**, 526–533.

Wang, D.-S., Kobayashi, T., Zhou, L.-Y., Paul-Prasanth, B., Ijiri, S., Sakai, F., Okubo, K., Morohashi, K.-I. & Nagahama, Y. (2007) Foxl2 up-regulates aromatase gene transcription in a female-specific manner by binding to the promoter as well as interacting with Ad4 binding protein/steroidogenic factor 1. *Molecular Endocrinology*, **21**, 712–725.

Wapstra, E., Olsson, M., Shine, R., Edwards, A., Swain, R. & Joss, J. (2004) Maternal basking behaviour determines offspring sex in a viviparous reptile. *Proceedings Of The Royal Society B-Biological Sciences*, **271**, S230–S232.

Wapstra, E., Uller, T., Sinn, D.L., Olsson, M., Mazurek, K., Joss, J. & Shine, R. (2009) Climate effects on offspring sex ratio in a viviparous lizard. *Journal of Animal Ecology*, **78**, 84–90.

Warner, D.A. & Shine, R. (2008) The adaptive significance of temperature-dependent sex determination in a reptile. *Nature*, **451**, 566–568.

Warner, D.A. & Shine, R. (2011) Interactions among thermal parameters determine offspring sex under temperature-dependent sex determination. *Proceedings Of The Royal Society B-Biological Sciences*, **278**, 256–265.

Warner, D.A., Uller, T. & Shine, R. (2009) Fitness effects of the timing of hatching may drive the evolution of temperature-dependent sex determination in short-lived lizards. *Evolutionary Ecology*, **23**, 281–294.

Weishampel, J., Bagley, D. & Ehrhart, L. (2004) Earlier nesting by loggerhead sea turtles following sea surface warming. *Global Change Biology*, **10**, 1424–1427.

Werren, J. & Beukeboom, L. (1998) Sex determination, sex ratios, and genetic conflict. *Annual Review Of Ecology And Systematics*, **29**, 233–261.

Wessels, S. & Hoerstgen-Schwark, G. (2007) Selection experiments to increase the proportion of males in Nile tilapia (*Oreochromis niloticus*) by means of temperature treatment. *Aquaculture*, **272**, S80–S87.

Weygoldt, P. (1980) Complex brood care and reproductive behaviour in captive poison-arrow frogs, *Dendrobates pumilio* O. Schmidt. *Behavioral Ecology And Sociobiology*, **7**, 329–332.

Witschi, E. (1929a) Studies on sex differentiation and sex determination in amphibians. III. Rudimentary hermaphroditism and Y chromosome in *Rana temporaria*. *Journal of Experimental Zoology*, **54**, 157–223.

Witschi, E. (1929b) Studies on sex differentiation and sex determination in amphibians. II. Sex reversal in female tadpoles of *Rana sylvatica* following the application of high temperature. *Journal of Experimental Zoology*, **52**, 267–291.

Witt, M.J., Hawkes, L.A., Godfrey, M.H., Godley, B.J. & Broderick, A.C. (2010) Predicting the impacts of climate change on a globally distributed species: the case of the loggerhead turtle. *Journal of Experimental Biology*, **213**, 901–911.

Yoshimoto, S., Okada, E., Umemoto, H., Tamura, K., Uno, Y., Nishida-Umehara, C., Matsuda, Y., Takamatsu, N., Shiba, T. & Ito, M. (2008) A W-linked DM-domain gene, DM-W, participates in primary ovary development in Xenopus laevis. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **105**, 2469–2474.

Zhang, F., Li, Y., Guo, Z. & Murray, B.R. (2009) Climate warming and reproduction in Chinese alligators. *Animal Conservation*, **12**, 128–137.

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Current position

2007 - PhD student, Dept. of Ecology and Evolution, University of Lausanne, Switzerland. Supervisor: Prof Nicolas Perrin

Genes, environment and sex: perspectives from ectothermic vertebrates

Education

10/2005 MSc Biology Swiss Federal Institute of Technology and University of Zürich, Switzerland.

Master thesis on MHC variability in Alpine ibex (*Capra ibex ibex*) populations. Supervisors: Prof. Lukas Keller and Prof Paul Schmid-Hempel

6/2001 Matura Type D (Spanish) at Gymnasium Interlaken (BE) Switzerland.

Research experience

12/2006 Research assistant (lab work)

Department of Ecology and Evolution, University of Lausanne. Supervisor: Prof. Claus Wedekind

6-8/2006 Research assistant (lab and field work)

Fish and Wildlife Biology, University of Montana, Missoula, USA

4,5/2006 Research assistant (field work)

Department of Behavioral Ecology, Universities of Neuchâtel, Switzerland and Calgary, Canada

2005-06 Research assistant (lab work)

Zoological Museum, University of Zürich, Switzerland

2003-04 Research assistant (lab and field work)

Department of Environmental Sciences, Swiss Federal Institute of Technology Zürich, Switzerland

KORA (Coordinated research projects for the conservation and management of carnivores in Switzerland), Berne, Switzerland

Institute of Plant Sciences, Swiss Federal Institute of Technology Zürich, Switzerland

Teaching experience

2007 -11 Undergraduate student teaching

Courses: Population genetics, Population dynamics, Marine Biology, Statistics, Experimental design, Botanics. BEC Masters program, University of Lausanne.

Languages

German (first language), English, French, Spanish (exchange year in Valdivia, Chile)

Skills

Molecular genetics: Fragment analysis, cloning & sequencing, CE-SSCP

Bioinformatics: R, UNIX (basics), WinBugs

Field: Telemetry, course in distance immobilisation

Publications

Grossen, C., Neuenschwander, S. & Perrin, N. (2011) Temperature-dependent turnovers in sex-determination mechanisms: a quantitative model. *Evolution*, 65, 64–78.

Jacob, A., Evanno, G., Siebenthal, Von, B.A., **Grossen, C.** & Wedekind, C. (2010) Effects of different mating scenarios on embryo viability in brown trout. *Molecular Ecology*, 19, 5296–5307.

Stöck, M.*, Horn, A.*, **Grossen, C.***, Lindtke, D., Sermier, R., Betto-Colliard, C., Dufresnes, C., Bonjour, E., Dumas, Z., Luquet, E., Maddalena, T., Sousa, H.C., Martinez-Solano, I. & Perrin, N. (2011) Ever-young sex chromosomes in European tree frogs. *PLoS Biology*, 9, e1001062.

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Oral presentations (related to this thesis)

2008 Sex determination in a changing environment

EES Summer School on 'The Evolution of Sex Chromosomes', Frauenchiemsee, Germany

2009 Turnover of sex-determination mechanisms induced by climatic change

CUSO workshop 'Evolution of sex-determination mechanisms'

2009 Sex chromosomes, sex reversal and climatic change

Dept of Ecology and Evolution, University of Lausanne

2011 Temperature-dependent turnovers in sex-determination mechanisms

Biology11, Annual Conference of the Swiss Zoological and Botanical Societies, Geneva

Grants and Prizes

2011 Award for best talk at Biology11, Annual Conference of the Swiss Zoological and Botanical Societies, Zürich

2008 Travel Grant to participate in a course in conservation genetics in portugal, Egalité des Chances

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