

Review



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Sex chromosomes as supergenes of speciation: why amphibians defy the rules?

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As reflected by the two rules of speciation (Haldane's rule and the large X-/Z-effect), sex chromosomes are expected to behave like supergenes of speciation: they recombine only in one sex (XX females or ZZ males), supposedly recruit sexually antagonistic genes and evolve faster than autosomes, which can all contribute to pre-zygotic and post-zygotic isolation. While this has been mainly studied in organisms with conserved sex-determining systems and highly differentiated (heteromorphic) sex chromosomes like mammals, birds and some insects, these expectations are less clear in organismal groups where sex chromosomes repeatedly change and remain mostly homomorphic, like amphibians. In this article, we review the proposed roles of sex-linked genes in isolating nascent lineages throughout the speciation continuum and discuss their support in amphibians given current knowledge of sex chromosome evolution and speciation modes. Given their frequent recombination and lack of differentiation, we argue that amphibian sex chromosomes are not expected to become supergenes of speciation, which is reflected by the rarity of empirical studies consistent with a 'large sex chromosome effect' in frogs and toads. The diversity of sex chromosome systems in amphibians has a high potential to disentangle the evolutionary mechanisms responsible for the emergence of sex-linked speciation genes in other organisms.

This article is part of the theme issue 'Genomic architecture of supergenes: causes and evolutionary consequences'.

1. Introduction

Reproductive isolation is the cornerstone of species formation, and the crucial role played by sex chromosomes is one of the best-accepted ideas in speciation research [1–5]. This is reflected by the two famous rules of speciation [6]: Haldane's rule [7]—the preferential sterility or inviability of hybrids of the heterogametic sex—and the large X-/Z-effect—the observation that X and Z chromosomes accumulate more incompatibilities than autosomes [8]. These rules trace back to the work of Darwin, who noted that the fitness of hybrids in interspecific crosses was often asymmetric (Darwin's corollary [9]), which was later linked to uniparentally transmitted elements like mtDNA or Y chromosomes [10,11].

A wealth of studies on mammals, birds and some insects have provided empirical support for both rules of speciation [2,12]. Accordingly, their strongly heteromorphic and partly non-recombining sex chromosomes are often considered as supergenes of speciation [13,14]. By contrast, many animal groups do not feature stable sex-determining systems, but instead evolved diverse sex chromosomes where gametologs (X/Y, Z/W) have remained undifferentiated and homomorphic [15]. Whether such sex chromosomes also qualify as supergenes of speciation remains an open yet key empirical question [16].

Many hypotheses have been proposed to explain why sex chromosomes should be hotspots of reproductive barriers at various stages of the speciation continuum. However, the two rules of speciation are composite phenomena, resulting from different causes in different contexts, which cannot be disentangled with heteromorphic sex chromosomes. To assess the generality of the role of sex-linked genes in reproductive isolation, and to understand the mechanisms generating the rules of speciation, one needs to focus on clades where sex chromosomes are still diverse [16]. Accordingly, a few recent studies suggested that the importance of sex chromosomes in speciation might not be as universal as previously assumed [17–19].

In this review, we summarize why differentiated sex chromosomes are expected to become supergenes of speciation, and why this assumption needs a reassessment when sex chromosomes are evolutionary labile and remain mostly undifferentiated, like in amphibians. Furthermore, we discuss how investigating the role of sex-linked genes in amphibian speciation can open novel perspectives to dissect the proximate bases of reproductive isolation.

2. Sex chromosomes as supergenes of speciation

(a) The evolution of sex chromosomes

Sex chromosomes evolve when a sex-determining mutation invades a population, so that individuals carrying the mutation develop into one sex (the heterogametic sex, i.e. XY males and ZW females), while the ones lacking the mutation develop into the other sex (the homogametic sex, i.e. XX females and ZZ males). A key step in the evolution of sex chromosomes is the arrest of recombination in the heterogametic sex soon after their establishment. The classic model of sex chromosome evolution has long explained the arrest of recombination by sex-antagonistic selection, favouring the tight linkage of sex-antagonistic genes with the sex-determining locus [3,20,21]. For instance, in an XY species (e.g. mammals), there is a selective advantage to keep male-beneficial alleles linked to the sex-determining locus (and suppress XY recombination in males), thus increasing the chance for these alleles to be transmitted to sons rather than daughters. Although widely accepted, this classical model has received little empirical support [22], and alternative hypotheses have been recently proposed to account for the recombination arrest of sex chromosomes (reviewed by [23,24]), such as early emergence of dosage compensation by regulatory X-Y divergence [25] or neutral accumulation of sequence divergence near the sex determiner [26].

When recombination stops, the Y/W non-recombining segment is expected to accumulate a high mutation load due to the combined effects of drift (four times higher than autosomes), selective sweeps (hitchhiking of deleterious mutations) and Müller's ratchet [21,27]. This load cannot be purged, as Y/W recessive mutations are never expressed due to their permanent state of heterozygosity. Over time, and potentially combined with X-Y/Z-W divergence caused by regulatory evolution [28], the Y/W chromosomes may lose most of their functional genes and degenerate. In parallel, hemizyosity facilitates positive selection on the X/Z chromosomes, which, combined with the enhanced drift resulting from their reduced effective population size (3/4 of autosomes), tends to make them evolve faster than the

rest of the genome, both in terms of sequence divergence and divergence in gene expression [29–33]. These processes are collectively known as the faster X- and faster Z-effects.

To sum up, the hemizyosity of sex chromosomes conveys specific characteristics that are expected to bolster their role in speciation compared to autosomes [1,4]: faster rates of evolution, strong linkage disequilibrium, the potential for sex-antagonistic genes and exposure of incompatible recessive alleles in hybrids. As we outline below, these features are theoretically expected to attract genes and supergenes that may play a key role in reproductive isolation along the continuum of divergence from populations to species.

(b) Sex chromosomes and pre-zygotic isolation

Early in the speciation process, diverging populations may adapt to distinct environments [34,35]. The genes involved in ecological differentiation are not expected to be disproportionately sex-linked, as disruptive selection is equivalent between sexes. Accordingly, the few genes underpinning ecological adaptations that have been identified so far in birds [36,37], mammals [38] and fishes [39] are autosomal. As they evolve faster, sex chromosomes may indeed diverge and fix new mutations more rapidly than autosomes, but the initial adaptation process may also depend on standing variation rather than novel mutations [40], in which case sex-linked genes should not be particularly involved.

Sex chromosomes have been proposed to play a prominent role in rapid sexual isolation [1,41] if they attract genes with sex-specific effects and sex-biased expressions, like those coding for primary (e.g. sexual organs, sperm-egg interaction proteins) or secondary sexual characters (e.g. ornaments and courtships) [42]. The evolution of these characters may be partly driven by sex-antagonistic arms races between males and females [43,44], which should be resolved more efficiently on the sex chromosomes [45]. For instance, recessive and dominant alleles at genes coding for, or *cis*-regulating, male traits are expected to accumulate on the X and Z chromosomes, where they will spend less and more time in males, respectively [46]. Sex-antagonistic gene conflicts are thus expected to favour sex linkage, and reciprocally, sex-antagonistic loci are predicted to recruit sex-determining genes [47]. Recently, Irwin [5] also highlighted that the elevated divergence on the Z chromosome of birds (due to faster Z-effects) should bolster positive selection for mutations involved in mating preferences.

Despite these assumptions, there is so far little empirical support that genes involved in pre-zygotic isolation accumulate on the sex chromosomes. Some meta-analyses found that sexually selected genes are non-randomly sex-linked [48], especially in fish [41], while others did not [49]. The genes responsible for mating discrimination (pheromones and courtship dances) between nascent species of *Drosophila* are found throughout the genome [50–54]. Similarly, in the Hawaiian cricket genus *Laupala*, song divergence and mating preferences are determined by multiple sex-linked and autosomal factors with small individual effects [55].

Therefore, sex chromosomes are expected to play a minor role in the early stages of divergence, but this role may increase if sexual conflicts promote sex linkage of sexual traits via sex-antagonistic selection. Clear empirical support for the latter hypothesis is still lacking though,

Table 1. List of the main genetic mechanisms proposed to involve sex-linked genes and supergenes in reproductive isolation, and whether they should or not apply in amphibians.

theory	description	requirement	evidence in animals	expectations in amphibians
<i>pre-zygotic isolation</i>				
sex-antagonistic genes	build up of genes and supergenes involved in sexual isolation	sex-linked reproductive traits and no recombination	weak	not expected
<i>intrinsic post-zygotic isolation</i>				
faster X-/Z-effect	faster divergence of X- or Z-linked loci	differentiated sex chromosomes	strong	not expected
dominance	hemizygoty of X- or Z-linked recessive incompatibilities in the heterogametic sex	differentiated sex chromosomes	strong	not expected
dosage compensation	disruption of dosage compensation	differentiated sex chromosomes	some	not expected
faster heterogametic sex	X-Y/Z-W interactions necessary for meiotic segregation and development of the heterogametic sex	—	some	potentially
meiotic drive	incompatibilities between sex-linked selfish distorters and their autosomal restorers	—	some	potentially
faster male	incompatibilities at fast-evolving male traits (notably spermatogenesis)	sex-linked male reproductive traits	some but not sex-linked	potentially, but not expected to be sex-linked
conflicts between sex-determining systems	incompatibilities (or sex ratio biases) caused by distinct sex-determining systems	turnover of sex-determining systems	rare	potentially, although the role in reproductive isolation is unclear
<i>reinforcement</i>				
co-adapted gene complexes	evolution of supergenes for species recognition to avoid the costs of hybridization	linkage between sex-linked hybrid incompatibilities, male traits and female preferences	some	not expected

especially as potential pre-mating genes, such as sexually selected genes, appear to be under autosomal control in many organisms.

(c) Sex chromosomes and post-zygotic isolation

As initially suggested by Dobzhansky [56] and Muller [57], intrinsic post-zygotic isolation is generally determined by genetic incompatibilities that reduce hybrid fertility and viability, resulting from the loss of complementary alleles at interacting genes as these underwent distinct evolutionary histories in diverging parental backgrounds. The stronger the divergence, the more genes become incompatible. Because sex chromosomes evolve faster than the rest of the genome (faster X- and Z-effects), they should accumulate more incompatibilities than autosomes [33]. Moreover, the Dobzhansky-Muller model implies that most incompatibilities are recessive. Due to dominance effects, for the same number of incompatible alleles, genes that are sex-linked trigger more problems in hybrids (through epistatic interactions

with other loci) as they are exposed to natural selection in hemizygous X/Z chromosomes.

An array of additional mechanisms of intrinsic post-zygotic isolation generated by genomic conflicts involving sex chromosomes has been proposed [12] (table 1). This includes rapid co-evolutionary arms races between X-/Z-linked sex ratio distorters and their autosomal suppressors ('meiotic drive theory' [58–60]), incompatibilities between interacting X-Y or Z-W genes necessary for proper meiotic segregation and sexual differentiation of the heterogametic sex ('faster heterogametic sex theory'; [61,62]), failure of epigenetic inactivation of sex chromosomes during meiosis [63,64], disruption of gene regulatory networks as necessary for dosage compensation in hybrids [8,65], or even activation of W-linked endogenous retroviruses [66].

Intrinsic post-zygotic incompatibilities have also been linked to the faster male hypothesis [67], which implies more incompatibilities affecting males than females, because male reproductive traits evolve faster than their female counterparts [68]. Reasons include more intense reproductive

competition in males and more sensitive spermatogenesis compared to oogenesis. In both cases, it naturally leads to stronger selection and faster divergence of male traits, many of which can be involved in reproductive isolation [67,69]. However, there is little empirical support that the genes coding for male-specific traits involved in post-zygotic isolation are disproportionately sex-linked [70]. As mentioned above (see §2(b)), sex-antagonistic selection can theoretically promote their recruitment on the sex chromosomes, increasing the influence of sex chromosomes in speciation, but this hypothesis has not been empirically demonstrated. Moreover, while faster male effects are typically invoked to explain Haldane's rule, it obviously would apply only to male-heterogametic taxa.

Whatever the proximate causes, there is ample support for the non-random build up and/or expression of intrinsic post-zygotic isolation on the X/Z chromosome (large X- and Z-effects), both regarding hybrid sterility and unviability. Evidence come from identification and genetic mapping of incompatibilities in hybrid crosses [71–73], as well as patterns of restricted gene flow at X- or Z-linked loci compared to autosomal loci across natural hybrid zones [74–76]. Indirect cues also lie in the genomic landscapes of differentiation between nascent species: sex chromosomes often bear higher genetic divergence compared to autosomes, which may reflect their role in speciation, among other (demographic) explanations [77,78]. Nevertheless, we note that the strong empirical support for the role of sex chromosomes in post-zygotic isolation essentially arises from crossing experiments in *Drosophila*, as well as genomic architecture of introgression and differentiation in mammals and birds, i.e. organisms with strongly differentiated sex chromosomes.

The various mechanisms hypothesized to explain Haldane's rule and the large X-/Z- effects are non-mutually exclusive, may confound each other and even interact to reinforce each other's. For instance, if male traits non-randomly build up on the sex chromosomes, selection on those traits should accelerate the divergence of X/Z sequences, which in turn increases the accumulation of exposed incompatibilities. Accordingly, rapid adaptive divergence and higher X-linked mutation rates were proposed to trigger faster X processes in *Drosophila* [79], hence causing the large X-effect popularized by this model organism [71]. For these reasons, very few studies have succeeded in dissecting the proximate causes underlying the rules of speciation [70,71], and the mechanisms explaining the contribution of sex-linked genes remain elusive.

(d) Sex chromosomes and reinforcement

When species come into contact before their genomes are fully incompatible, selection against hybridization generated by post-zygotic isolation may reinforce assortative mating through the establishment of co-adapted gene complexes like supergenes promoting species recognition [80–82]. In the animal kingdom, mate recognition may encompass visual, acoustic or olfactory cues, and implies the coevolution of genes coding for species-specific traits, and preferences for these traits. Hence, the genes controlling these traits are expected to build around the genes causing low hybrid fitness, to limit the cost of interbreeding [80,82,83].

Since they facilitate sex-antagonistic selection, recombine less than the rest of the genome and are disproportionately responsible for hybrid sterility and inviability (see §2(c)), sex chromosomes thus make suitable hosts for species-

recognition complexes, by offering linkage between the genes coding for preferences, traits and hybrid incompatibilities. In turn, theoretical models suggest that sex linkage of the traits driving both pre- and post-zygotic isolation may promote adaptive speciation [80,83], and such associations were even demonstrated in a few female heterogametic organisms, including insects [84] and birds [85].

Sex chromosomes may thus also be involved in later stages of the speciation process, as selection against hybrids directly leads to the evolution of fine-tuned species-recognition systems in sympatry, where the relevant traits are sheltered from recombination.

3. The peculiar sex chromosome evolution of amphibians

(a) Amphibian sex chromosomes frequently change

Unlike mammals, birds and other famous speciation models (*Drosophila*, Orthoptera), amphibians (as many fishes and reptiles) rarely exhibit strongly differentiated sex chromosomes [15]. All species investigated (mostly anuran amphibians: frogs and toads) have a genetic control of sex determination (even though it is not always strict, [86]), with male or female heterogamety [87–89]. Because amphibians lack a degenerated Y or W, evolutionary biologists have often considered their sex chromosomes to be nascent, i.e. in their early stages of differentiation. In fact, the absence of strong karyotypic and genetic differences between the amphibian X and Y, or Z and W, has long precluded their identification in many species groups [15].

Over the past decade, extensive research on a few families (notably Hylidae and Ranidae) has identified the sex-determining system of several taxa and characterized putative mechanisms that prevent Y- and W-chromosome decay. These studies found recurrent transitions of sex chromosomes, with remarkable differences among closely related species (sometimes even populations of the same species) [90–93]. The shifts are not always random: the same few chromosome pairs have been regularly co-opted, presumably as they carry genes on top of the conserved sex-determination cascade of vertebrates [91,93,94]. Recurrent changes of the sex chromosome systems would then allow purging of the mutation load that accumulates on the Y/W during periods of non-recombination, by restoring it as an autosome [95]. Moreover, it was suggested that the pattern of male heterogamety was generally conserved throughout the transitions, presumably due to a strong heterochiasmy (males generally recombine far less than females [93,96,97]) that may facilitate the spread of new sex chromosomes [91]. Nevertheless, changes in heterogamety (from XY to ZW and vice-versa) are still frequently observed between sister lineages [92,93,98].

(b) Amphibian sex chromosomes frequently recombine

The reduced male recombination in amphibians does not spare their sex chromosomes. In frogs, high-density linkage mapping has shown that X and Y chromosomes only recombine at the tips in males, which is necessary for proper meiotic segregation [97,98]. However, there is ample phylogenetic and phylogeographic evidence that sex chromosomes occasionally recombine, to the extent that gametologs do not strongly differentiate.

In male-heterogametic systems, phylogenetic studies on Hylidae and Bufonidae highlighted that for markers distributed at various places along the sex chromosomes, alleles cluster by species rather than by gametologs [92,99]. This implies some gene flow between the X and Y chromosomes after the divergence of the corresponding species (otherwise the X alleles of different species would form one branch, while the Y alleles would form another branch). In fact, in *Hyla* tree frogs, the only locus where X and Y alleles diverged prior to the species diversification is exon 1 of the candidate sex-determining gene *dmrt1*, while adjacent sequences (approx. 20 kb apart) already showed phylogenetic cues of recombination [100]. Coalescent analyses of sex-linked microsatellite markers were similarly consistent with non-zero X-Y recombination rates [101], which, although low (10^5 times smaller than X-X recombination), were theoretically sufficient to maintain undifferentiated sex chromosomes free of deleterious mutations [102].

How can X and Y chromosomes recombine if the males that carry them do not? Because amphibian sex determination is not always strictly genetic, but still partially depends on environmental and random factors, X-Y individuals may sometimes develop into females under particular conditions (notably temperature) or sex-linked genotypes (weakly masculinizing Y alleles), in which case sex chromosomes are free to recombine as in regular X-X females [103,104]. Empirical evidence of these hypotheses come from the common frog *Rana temporaria*, where sex-reversed X-Y individuals feature high female-like recombination rates, including at sex-linked markers [86]. Therefore, occasional sex-reversal can produce bursts of recombined Y haplotypes, hence maintaining sex chromosomes in a perpetual stage of early divergence [103]. Other forms of leaky genetic sex determination (e.g. developmental noise [104]) allow sex-linked genes to behave (and recombine) like autosomal genes during a few generations [105]. In addition, X-Y divergence and male recombination rates can show high intraspecific variation, as in *H. arborea*, implying that males may also directly recombine their X-Y chromosomes [106]. The non-recombining Y segment can thus be very short, to the point where high-throughput sequencing is required to reveal sex-linked polymorphisms (e.g. *Bombina*, [107]).

Patterns of sex chromosome differentiation with female heterogamety have been less documented. These systems are less frequent across the amphibian tree of life [15,91], and ZW chromosomes are more difficult to identify, presumably because female recombination homogenizes Z and W haplotypes. For instance, only a single sex-linked marker was found in the female-heterogametic common toad *Bufo bufo*, while hundreds of XY markers were identified in its male-heterogametic sister species *B. spinosus* [98]. Non-recombining segments in amphibians are usually shorter in ZW compared to XY species. In *Hyla*, the strong heterochiasmy was conserved throughout the evolution of Western Palaearctic species, irrespective of the heterogametic system: X and Y gametologs recombine only at chromosome tips (due to low recombination in males), while Z and W gametologs recombine over the entire chromosome (due to high recombination in females) and consequently remain differentiated only near the sex-determining region [93]. As such, the non-recombining segments varied by two orders of magnitude: spanning less than 3 centimorgan (cM) and greater than 200 cM for the ZW and XY chromosomes, respectively [93].

Because most species possess homomorphic sex chromosomes that sometimes remain entirely undifferentiated except near the sex determiner, frogs are not expected to have evolved dosage compensation, a prediction verified by a few studies [108]. However, it was hypothesized that frogs may use sex chromosome-wide facultative heterochromatin mechanisms to regulate dosage in species with well-differentiated sex chromosomes [108]. Relatively little is known on frog epigenetics, but the occurrence of heteromorphic sex chromosomes in a few species groups would provide insights into the evolution of dosage tolerance versus compensation during sex chromosome evolution [108].

Overall, the available research indicates that amphibian sex chromosomes generally remain poorly differentiated, due to high ZW female recombination in female-heterogametic species and occasional XY recombination in male-heterogametic species.

(c) Amphibian sex chromosomes are not hotspots of sex-antagonistic genes

The specificities of the amphibian sex chromosomes highlighted above suggest no support for a role of sex-antagonistic genes in their evolutionary dynamics [22]. Amphibian sex determination is leaky, sex chromosome recombination depends on the phenotypic sex (not the heterogametic sex), and the Y lacks divergence strata that would have been expected if recombination arrests had accompanied the successive recruitment of sex-antagonistic genes alongside the sex determiner (the main assumption behind the classical model of sex chromosomes evolution, §2(a)).

Moreover, female-heterogametic species, in which Z and W recombine intensely, seem to display the same level of sexual dimorphism as male-heterogametic species, suggesting that sexual dimorphism does not rely on sex-limited genes [93]. The same argument can be made from male-heterogametic species where individuals are polymorphic in terms of X-Y recombination and differentiation, as in *R. temporaria* [105,109]. If sex-antagonistic genes were sex-linked, the lower X-Y recombination (and higher X-Y differentiation) should increase the fitness of males and females. Instead, phenotypic studies found equivalent morphological characteristics and reproductive outputs between males bearing differentiated and undifferentiated Y chromosomes, which argues against the fixation of male-beneficial/female-detrimental alleles on the Y [110]. Similarly, transcriptome analyses revealed similar expression profiles regardless of X-Y differentiation, while the genes with sex-biased expressions were uniformly distributed across the genome [111,112].

Altogether, these studies suggest that sexual traits are not disproportionately controlled by sex-linked genes in amphibians, and that instead, genes with sex-antagonistic effects are mostly autosomal. As discussed earlier, this assumption might extend to all major vertebrate clades [25,26,28].

4. Sex chromosomes as supergenes of speciation in amphibians?

(a) Pre-zygotic isolation

As lineages diverge, pre-mating barriers may build up through the divergence of sexual traits, which in theory could evolve rapidly on the sex chromosomes via sex-

antagonistic selection (§2(b)). However, sex-antagonistic genes are mostly autosomal in amphibians (§3(c)) and should thus not generate a disproportionate role of sex chromosomes in speciation.

In addition, primary sexual differentiation may not be as relevant for reproductive isolation as it is in other animals (e.g. insects), since fertilization is external in the vast majority of species. Instead, post-mating pre-zygotic isolation may mainly involve sperm–egg incompatibilities. Experimental studies in frogs found female variation in egg protein composition affecting sperm motility, swimming velocity, capacitation and acrosome reaction, thus affecting fertilization [113]. Gamete recognition genes are among the fastest-evolving genes of the genome and have been proposed to explain the rapid diversification of marine species groups with external fertilization [114]. In mammals, the pair of key genes involved in sperm–egg recognition are located on autosomes [115]. In amphibians, sperm-incompatibility interactions have never been studied in a speciation context, so the genetic factors involved, including their chromosomal locations, remain entirely unknown.

Likewise, we know very little on the genetic architecture of traits potentially involved in pre-mating barriers, such as ecological differentiation and/or mate choice. Speciation is driven by the rapid evolution of such signals yet seems to be rare in amphibians [116]. A significant literature has suggested that differences of mating calls, coloration or ecology may cause speciation between genetically close lineages (by ‘magic traits’ [117]), but several of these cases have been recently challenged, as the corresponding traits did not enforce strong reproductive isolation, or the species primarily evolved through other processes. For instance, in Malagasy frogs, adaptive radiations may have been initiated in allopatry, despite their ecological divergences that now enable sympatry [118,119]. In poison frogs, lineage-specific colour differences proposed to prevent interbreeding [120] appear insufficient to enable effective assortative mating in sympatry [121]. The high speciation rates in this group nevertheless still associates with their diversity in coloration and the role it plays in aposematic mimicry [122,123]. In fire salamanders, the genetic structure between populations inhabiting distinct habitats was hypothesized to reflect nascent ecological speciation [124], although the pattern can be explained by isolation-by-distance [125]. In European amphibians, hybrid zone analyses suggest that speciation proceeds from genomic divergence in allopatry, generating post-zygotic barriers between cryptic species long before the divergence of sexual traits responsible for pre-mating isolation in sympatry [126,127] (see also §4(c)). Hence, speciation by the rapid build up of pre-zygotic isolation does not seem common in amphibians, and sex chromosomes are not expected to play a major part in it.

(b) Post-zygotic isolation

The main tenet behind the various mechanisms involving sex-linked genes for the interpretation of Haldane’s rule and the large X-/Z-effect is the hemizygoty of the X or Z chromosome in the heterogametic sex [1,4] (see §2(c)). In amphibians, however, these chromosomes are rarely hemizygous: occasional or pervasive recombination have prevented X-Y and Z-W differentiation (see §3(b)). As a consequence, X or Z sequences are not expected to display higher rates of

evolution compared to autosomes (faster X-/Z- effects), nor did they evolve dosage compensation and sheltered genes with sex-specific effects (see §§3(b) and (c)). Hence, several of the hypotheses invoked to explain the rules of speciation do not apply for the undifferentiated sex chromosomes of amphibians: these should not disproportionately cause intrinsic post-zygotic isolation by hemizygous exposure of recessive incompatibilities (the ‘dominance’ effect of Haldane’s rule), by disrupting dosage compensation, nor by the accumulation of sex-antagonistic genes that may in turn cause faster male effects (table 1).

The proportion of sex-linked versus autosomal hybrid genetic incompatibilities has rarely been quantified in amphibians. Most empirical data come from the analysis of secondary contact zones where diverging lineages admix. Comparisons in the level of introgression between autosomal and sex-linked markers via geographic cline analyses accordingly found little support for large X-effects. In green toads (*Bufo*), both types of markers behave similarly across the porous hybrid zone between two *B. viridis* lineages in north Italy (*B. v. viridis/balearicus*), as well as across the narrow hybrid zone between *B. viridis* and *B. boulengeri* in Sicily [128]. In common frogs, analyses of species-diagnostic single-nucleotide polymorphisms mapped on a reference genome revealed a homogeneous landscape of introgression between the sister species *R. temporaria* and *R. parvipalmata*, with the sex chromosomes actually admixing slightly more than the average autosome [127]. Remarkably, even the loci located near the presumed sex-determining gene (*Dmrt1*) featured similar levels of introgression as the rest of the genome [127].

The only support for large X-effects in amphibians comes from the male-heterogametic *Hyla* tree frogs, where replicate hybrid zone studies showed significantly reduced introgression at sex-linked markers for two pairs of species [17,127]. Among possible mechanisms that could apply to homomorphic sex chromosomes, we can hypothesize X-Y incompatibilities disrupting meiosis in the heterogametic sex (faster heterogametic sex), or incompatibilities between selfish genetic elements and their suppressors (meiotic drive). In addition, although sex chromosomes are clearly homomorphic in *Hyla* [129], the hybridizing species studied harbour Y haplotypes that are on a path to differentiation, due to regionally reduced X-Y recombination [106]. It is thus possible that the X chromosomes have momentarily become slightly hemizygous, with the potential to have caused dominance or faster X-effects in the contact zone [127].

While two-thirds of investigated amphibians seem to follow Haldane’s rule [2], especially for inviability, this result essentially comes from a single study on Bufonidae [130], where all species were assumed to be female heterogametic. We now know that this is not the case in all Bufonidae genera, where male heterogamety is also common (e.g. *Bufo* [98]; *Bufo* [99]). Moreover, asymmetric patterns of cytonuclear introgression between XY and ZW *Bufo* species (see §4(d)) run against Haldane’s predictions [98]. In urodeles, asymmetric reduction of survival in hybrids between male-heterogametic newts (*Triturus*) suggested cyto-nuclear incompatibilities rather than sex-linked genes to explain Haldane’s rule [131]. In ZW *Xenopus* frogs, an elegant experimental design involving sex reversal and interspecific crosses revealed that hybrid incompatibilities disproportionately caused sterility in phenotypic males more than in phenotypic females, irrespective of their genetic sex (ZZ or

ZW genotypes) [70]. While opposing Haldane's pattern, these results demonstrate faster male processes caused by autosomal genes [70]. Hence, Haldane's rule in amphibians may not be as universal as assumed across the animal kingdom, and it certainly needs substantial reevaluation.

Overall, the (scarce) empirical data so far available on amphibians thus suggests little role for sex-linked genes in intrinsic post-zygotic isolation. Genes causing sex-specific effects on hybrid fitness (e.g. faster male factors) are seemingly autosomal, and the lack of hemizygoty should not bolster more sex-linked compared to autosomal incompatibilities. By contrast, the curious exceptions identified in *Hyla* could indicate that speciation genes become increasingly sex-linked under certain evolutionary conditions, for instance, when X and Y start to differentiate.

(c) Reinforcement

For the same reasons, amphibian sex chromosomes are not predicted to harbour an excess of genes involved in pre-zygotic (§4(a)) and intrinsic post-zygotic isolation (§4(b)), neither should they contribute to the build up of gene complexes underlying pre-mating barriers as a response to selection against hybrids (reinforcement). Indeed, species-specific preference traits that would help escaping the hybridization load should not disproportionately become sex-linked if the sex chromosomes do not offer tighter linkage and more hybrid incompatibilities than autosomes.

Moreover, reinforcement has been rarely demonstrated in amphibians. The few convincing cases involved character displacement of advertisement calls in Australian tree frogs [132], or coloration in poison frogs [122] and newts [133]. By contrast, several attempts to demonstrate reinforcement were inconclusive. For instance, in the classical fire-bellied toad model (*Bombina*), ecologically and phenotypically distinct species hybridize in narrow contact zones [134], but their call properties shift gradually along species transitions, without causing pre-mating isolation [135]. The genetic architecture of anuran mating calls is virtually unknown, and mapping the genes involved would thus be a first step towards understanding whether they are sex-linked and if they are susceptible to reinforcement. The same applies for the pre-mating behaviour of urodeles (newts and salamanders), which rely on courtship dances and pheromone exchanges. Cryptic factors like extraspectrum coloration (including biofluorescence) and olfactory cues that may be involved in amphibian mate selection [136,137] have remained mostly overlooked in the context of speciation [138].

To conclude, even if reinforcement was more common than currently suggested by the literature, we would not expect it to involve sex chromosomes more than autosomes, since there is neither empirical evidence nor theoretical predictions that genes coding for species-specific traits or preference should be overrepresented on sex chromosomes relative to autosomes in amphibians.

(d) Contributions of sex chromosome turnovers

Given their lability in sex determination, closely related amphibian lineages with different sex chromosome systems may come into secondary contact. One could expect that carrying non-homologous sex chromosomes should enhance reproductive isolation and speciation. Mixing different sex

chromosome systems should trigger genic conflicts in hybrids, drastically altering their sex determination and gametogenesis, and thus causing intersex and sterile individuals, respectively. Alternatively, different sex chromosome systems may remain compatible in hybrids if one supersedes the other, or if their co-contributions to sex determination ensure balanced sex ratios and sex-specific fitness. In fish, perhaps the only vertebrate group where sexually selected traits seem to often be sex-linked [41], the evolution of neo-sex chromosomes has driven inter-species phenotypic divergence at traits responsible for reproductive isolation, in turn triggering speciation [139,140]. However, we have seen that such situations are less likely in amphibians, since sexual dimorphism seems to be largely under autosomal control (see §3(c)).

The best-documented case of variation in sex chromosomes among closely related populations is the Japanese wrinkled frog *Glandirana rugosa*, where geographic forms bearing homologous XY and ZW chromosomes meet in secondary contacts [90,141]. Experimentally produced F1 progenies showed balanced sex ratios, as YZ and XW embryos developed into males and females as expected, while 'conflictual' XZ and YW genotypes alternatively developed into either sex [141]. Only WW and YY genotypes obtained by backcrossing showed hybrid breakdown due to the exposure of recessive deleterious mutations, proportional to their respective state of differentiation. Specifically, the W chromosome of *G. rugosa* shows signs of decay, and all WW died after hatching. By contrast, the Y chromosome is presumably younger and YY offspring reached maturity, although fewer than in control crosses [141].

A similarly promising system was recently documented in the common toads *Bufo bufo* (ZW) and *B. spinosus* (XY), which form narrow hybrid zones in France maintained by intrinsic post-zygotic incompatibilities [98]. Moreover, the strong asymmetry in mitochondrial introgression is consistent with the hypothesis that the ZW system dominates the XY system, skewing sex ratios among hybrid genotypes (namely that all XZ hybrids develop as males and all YW develop as females), although alternative explanations (range shifts) could also explain the pattern of mitochondrial admixture [142].

These rare cases emphasize how colliding sex chromosome systems could in principle contribute to speciation by altering sex determination in hybrids. Yet, it remains unclear to what extent the changes of sex chromosomes contribute to reproductive barriers, since species boundaries have remained porous.

5. Perspectives for speciation research

(a) Dissecting the genomic bases of speciation

One way to disentangle the many hypotheses that link sex chromosomes to speciation (table 1) is to quantify the effect of sex-linked genes in situations where some hypotheses should apply, and others should not. As we have summarized, most of the isolating mechanisms involving sex-linked genes stem from the high divergence between gametologs, recombination arrest between them and their putative accumulation of sex-antagonistic genes—the latter assumption being increasingly questioned [22,25,26]. As these three properties are all ubiquitous in organisms with highly

Table 2. Potential mechanisms of genetic incompatibilities in respect to the rules of speciation in species bearing homomorphic sex chromosomes like amphibians (Haldane's rule: asymmetry of hybrid fitness; large sex chromosome effect: reduced introgression at sex-linked compared to autosomal loci). Other mechanisms may include autosomal–autosomal interactions or cyto-nuclear interactions.

		large sex chromosome effect		
		yes		no
XY system	Haldane's rule	yes	faster male/faster heterogametic sex/meiotic drive	faster male (autosomal)
		no	other	other
ZW system	Haldane's rule	yes	faster heterogametic sex/meiotic drive	other
		no	potentially faster male (sex-linked)	potentially faster male (autosomal)

differentiated sex chromosomes, clades that offer a variety of sex-determining systems and degrees of sex chromosome differentiation are invaluable for a proper dissection of the mechanisms prevailing behind the rules of speciation.

Extending beyond amphibians, for the same amount of divergence, reproductive isolation appears generally weaker without highly differentiated sex chromosomes. In *Anopheles* mosquitoes, where X and Y chromosomes are highly heteromorphic, hybrid males suffer from strongly depressed sterility and viability, while in *Aedes* mosquitoes, where X and Y chromosomes are homomorphic, hybrid males only suffer from limited sterility [16]. A meta-analysis also corroborated the view that post-zygotic incompatibilities expressed in F1 hybrids are stronger in species with heteromorphic than with homomorphic (or no) sex chromosomes [143] (but see [144]). In turn, the speciation clock may tick slower in squamate species lacking heteromorphic sex chromosomes [145]. Taken together, the literature thus indirectly pleads for the dominance and/or faster X/Z hypotheses as the main factors behind sex-linked incompatibilities: when these factors are lacking, as in organisms with undifferentiated sex chromosomes like amphibians, post-zygotic isolation evolves slower and the large X-/Z-effect is not conspicuous.

Sex chromosomes may still behave like supergenes of speciation even without strongly heteromorphic sex chromosomes, due to the potential role of faster heterogametic sex effects or nascent dominance/faster X-effects, e.g. when Y haplotypes have not recombined for some generations as in *Hyla* (§4(b)). Furthermore, the rapid species-specific degeneration of Y-linked genes and associated adjustment of expression of X-linked gametologs by dosage compensation may also lead to a rapid build up of sex-linked species incompatibilities [19]. This would account for large X-effects reported in closely related *Silene* plants, where the young Y chromosome degenerated in one species but not in another [18].

Without the confounding effects of dominance and faster X-/Z-effects, one can assess the contributions of alternative mechanisms of intrinsic post-zygotic isolation, like the faster male, meiotic drive and faster heterogametic sex models. These hypotheses do not make assumptions on sex chromosome differentiation and imply different expectations on the rules of speciation depending on heterogamety. Female-heterogametic systems in particular have a high potential for dissecting among these mechanisms: faster heterogametic-sex effects and meiotic drive should disproportionately affect fitness in the heterogametic sex (ZW

females) while faster male should rather affect the homogametic sex (ZZ males). Hence, Haldane's and large Z-effects would imply the meiotic drive and faster heterogametic sex hypotheses, but would be inconsistent with the faster male hypothesis. Table 2 provides a key to the observations of the rules of speciation in species bearing undifferentiated sex chromosomes, with respect to the mechanisms potentially involved. Contrasting between the inviability and sterility aspects of Haldane's rule would also be informative, as these may not be caused by the same genetic mechanisms [2].

Finally, we stress that the large X-/Z-effect deserves a new terminology when it involved truly undifferentiated sex chromosomes. The original expression, which reflects the role of the X/Z chromosome, implies hemizyosity (due to the degenerated nature of the Y/W) as the underlying cause of sex-linked incompatibilities. When alternative mechanisms that are independent of hemizyosity apply (such as faster male, meiotic drive, and faster heterogametic sex mechanisms), it would be more accurate to refer to a 'large sex chromosome effect', since both sex chromosomes are equally responsible for the incompatibilities.

(b) Consequences on the mode of speciation

If the amphibian sex chromosomes are not supergenes of speciation, this should impede the establishment of reproductive isolation [143,145]. Accordingly, amphibian species form at significantly lower rates compared to vertebrates where sex chromosomes are strongly heteromorphic like in mammals and birds [146,147]. By reducing opportunities for pre-mating barriers and reinforcement (which can theoretically boost speciation rates [80]) early in the speciation process, the putative lack of sex-linked supergenes could thus explain why amphibian reproductive isolation rather evolves gradually by post-zygotic incompatibilities [116,127].

By contrast, however, African cichlid fishes, which are famous for their explosive rates of adaptive speciation [148], also display high rates of sex chromosome turnovers involving mostly homomorphic sex chromosomes [149,150]. Whether the rapidly changing undifferentiated sex chromosomes of cichlids contributed to their rapid divergence and reproductive isolation remains to be established (see also [140]). Yet the striking differences in the paces of speciation between amphibian and cichlids suggest that the link between reproductive isolation and the nature of sex chromosomes is difficult to establish.

The absence of strong pre-mating barriers in many amphibians offers opportunities for hybridization between

deeply diverged lineages, which can be a source of novel diversity [151–153]. As they inherit strongly differentiated, partly incompatible genomes, such hybrids face meiotic issues that need to be circumvented in order to perpetuate. To do so, hybrid taxa often rely on peculiar mechanisms like hybridogenesis (e.g. *Pelophylax* [154,155]) and allopolyploidization (e.g. *Bufo* [156,157]), which both involve the clonal transmission of one parental genome [156,158]. Undifferentiated sex chromosomes play a fundamental role in these processes, by conditioning sex determination in hemiclinal hybrids through the directionality of the initial crosses, which feeds back to the sex ratio and fitness of possible backcrosses, hence the evolutionary fate of the hybrid taxa [159]. This complex matter was recently reviewed in a previous issue of this journal [159], and we will provide here one compelling case that illustrates how the lack of sex chromosome differentiation can open the path towards hybrid speciation, namely the hybridogenetic water frog system (genus *Pelophylax*). These frogs are male heterogametic [155], and the hybrid taxon *P. esculentus* (genomes RL) was presumably formed by initial crossing between females *P. ridibundus* (genomes RR) and males *P. lessonae* (genomes LL). Hence, the Y chromosome is inherited with the L genome, while the X chromosome is inherited with the R genome. But because it has been transmitted clonally by *P. esculentus* individuals (which need to mate with *P. lessonae* to perpetuate) in many populations, the R genome accumulated deleterious mutations, so RR progenies (all XX females) obtained by inter-hybrid crosses are not viable. Importantly, this system can only be maintained because the XY sex chromosomes of *Pelophylax* are undifferentiated: otherwise, *P. esculentus* would inherit both a clonal X and a degenerated Y, a putatively lethal situation. Therefore, while the undifferentiated sex chromosomes of amphibians may contribute little to most instances of speciation, their plasticity offers opportunities to generate and sustain new species of reticulate origin.

6. Conclusion and future directions

Sex chromosomes play a large role in reproductive isolation, principally as they generate post-zygotic incompatibilities more rapidly than do autosomes and have thus been considered as supergenes of speciation. In particular, the highly differentiated sex chromosomes of mammals and birds may also facilitate the build up of species-recognition systems, by linking the traits involved in pre-mating (sexually selected genes) and post-mating isolation (hybrid incompatibilities). Instead, the homomorphic sex chromosomes of amphibians (so far having been essentially studied in anurans, i.e. frogs and toads) do not play by these rules, and their influence on speciation is more typically similar to that of autosomes.

Amphibians then constitute an opportunity to dissect the genetic mechanisms of reproductive isolation involving sex-linked genes. The strong variability of sex-determination systems documented in frogs and toads combined with the numerous secondary contacts between pairs of lineages at different stages of divergence has great potential for comparative studies. In particular, new insights will come from assessing the two rules of speciation (Haldane's rule and the large X-/Z-effect) in phylogenetically resolved groups that exhibit variation in heterogamety (XY and ZW systems), and where sex chromosomes are strictly undifferentiated, or

on the path of differentiation and even decay. For instance, the genus *Hyla* and *Glandirana* will remain promising models as some lineages evolved heteromorphic sex chromosomes [141,160]. At the other end of the spectrum, the loss of genetic sex determination in some rapid populations but not others (e.g. *R. temporaria* [22]) could allow quantification of the strength of reproductive barriers with and without sex-linked genes.

We pinpoint three major advances that would promote amphibians as a research model to dissect the role of sex chromosomes as supergenes of speciation. First, because they are often homomorphic, sex chromosomes have been insufficiently characterized, and basic knowledge such as the heterogametic system is known in less than 5% of amphibian species [15]. Due to their large genomes, overlooked diversity and life-history traits (longer generation times, fewer offspring) that make them less easy to breed, caecilians and urodeles have only received preliminary assessments [144]. These groups presumably feature a high diversity of homomorphic but also heteromorphic sex chromosomes [144], hence a great comparative potential. Thankfully, new genotyping and bioinformatic tools now allow screening for sex-linked polymorphism with high-throughput sequencing data without the need for crossing experiments [161,162]. Second, in addition to identifying the sex chromosomes, genomic analyses should also measure molecular differentiation between gametologs (hence Y/W degeneration), which would also offer frameworks for studies on dosage tolerance [108]. High-quality genome assemblies and bioinformatic developments enabling phasing of sex-linked haplotypes and quantification of the accumulation of repetitive elements will be decisive for such studies [163,164]. Third, genome assemblies should become increasingly available in order to map the loci contributing to reproductive barriers, in turn allowing testing for large sex chromosome effects, which has so far been limited to a few organismal groups that benefit from reference genomes.

Finally, the increased interest for amphibian sex determination and speciation will inevitably lead to the discovery of more hybridizing species pairs with distinct sex chromosome systems [98,141]. These peculiar cases will not only be useful to understand whether there is a link between sex chromosome dynamics and speciation rates. They may also shed light on the proximate mechanisms of sex determination, by revealing how independently evolved systems interact to affect the sex-determining cascade. Reciprocally, unravelling the sex chromosomes in closely related species will be key to assessing the potential role of hybridization in sex chromosome evolution. New sex-determining systems were shown to emerge through Y introgression in fishes [163], a mechanism that has so far been overlooked in amphibians, although it may contribute to their tremendous diversity of sex chromosomes.

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All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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