Chapter 18
Polyploidization and Sex Chromosome Evolution in Amphibians

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Abstract Genome duplication, including polyploid speciation and spontaneous polyploidy in diploid species, occurs more frequently in amphibians than mammals. One possible explanation is that some amphibians, unlike almost all mammals, have young sex chromosomes that carry a similar suite of genes (apart from the genetic trigger for sex determination). These species potentially can experience genome duplication without disrupting dosage stoichiometry between interacting proteins encoded by genes on the sex chromosomes and autosomal chromosomes. To explore this possibility, we performed a permutation aimed at testing whether amphibian species that experienced polyploid speciation or spontaneous polyploidy have younger sex chromosomes than other amphibians. While the most conservative permutation was not significant, the frog genera *Xenopus* and *Leiopelma* provide anecdotal support for a negative correlation between the age of sex chromosomes and a species' propensity to undergo genome duplication. This study also points to more frequent turnover of sex chromosomes than previously proposed, and suggests a lack of statistical support for male versus female heterogamy in the most recent common ancestors of frogs, salamanders, and amphibians in general. Future advances in genomics undoubtedly will further illuminate the relationship between amphibian sex chromosome degeneration and genome duplication.

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18.1 Introduction

Why polyploidization is more common in plants than in animals is a central question in biology (Mable 2004; Muller 1925; Orr 1990), and multiple explanations have been put forward (reviewed in Gregory and Mable 2005; Mable 2004; Orr 1990; Otto and Whitton 2000). One possibility is that the propensity for a species to undergo polyploidization is related to the extent of sex chromosome degeneration. Sex chromosome degeneration is the evolution of differences in gene content that goes beyond the fundamental difference in the presence or absence of a genetic trigger for sex determination. Degenerate sex chromosomes could present problems during "diploidization" of a polyploid genome. Diploidization refers to the phenomenon by which a polyploid species transitions to a mode of chromosomal inheritance that is similar to a diploid species. A key feature of diploidization is the switch from polysomic inheritance, where multivalents form during cell division, to disomic inheritance, where only bivalents form (Wolfe 2001). This phenomenon is probably achieved via divergence between duplicated pairs of homologous chromosomes. Diploidization therefore could be instantaneous when polyploidization occurs via allopolyploidization (genome duplication associated with hybridization among diverged species) because duplicated homologous chromosome pairs already diverged from one another in the ancestral species. When a polyploid genome with duplicated sex chromosomes becomes diploidized, one pair of sex chromosomes presumably begins to segregate autosomally. With a degenerate Y-chromosome, for instance, the nascent autosomal pair that was previously a pair of sex chromosomes would initially have three possible genotypes: A_XA_X, A_X0, and 00 where A_X refers to an autosomal allele derived from an ancestral X-chromosome and 0 refers to a missing allele that was lost on the ancestral Y-chromosome. If the 00 genotype is deleterious or lethal, there would be reproductive incompatibilities in the early stages of diploidization until the degenerate chromosome is lost. This fitness cost could be mitigated if a functional paralogous allele were still present on the sex chromosomes, as would be expected in an autopolyploid (formed from genome duplication within a species). In allopolyploids, however, sex chromosomes could degenerate in unique ways in each ancestral species, giving rise to diverged gene content, so homozygous null genotypes would be a bigger problem. In both types of polyploids, dosage balance requirements—natural selection favoring a specific relative expression level of interacting genes (Papp et al. 2003; Qian and Zhang 2008)—could impose a fitness cost on a homozygous or heterozygous null autosomal genotype in a polyploid.

Polyploidization could also present challenges to species with degenerate sex chromosomes that have also evolved mechanisms for dosage compensation (Orr 1990). Dosage compensation equilibrates expression levels of genes that have one allele in one sex and two alleles in the other sex (for example, X-linked genes have one allele in XY males but two alleles in XX females). In this way the stoichiometry of expression of X-linked and autosomal genes is constant, or "balanced", in males

and females. Orr (1990) argued that a polyploid lineage would initially be established via backcrossing a new polyploid individual to diploid individuals, and that this would disrupt this balance.

The evolution of differences in gene content is a combined consequence of the migration of genes, especially genes with sex-specific function, to one or the other sex chromosome, and also the loss of genes from the region of suppressed recombination on the sex-specific sex chromosome (for example, the Y-chromosome). The disparity in gene content between the sex chromosomes is thought to increase over time as a consequence of natural selection (Bergero and Charlesworth 2009; Charlesworth et al. 2005). Substantial disparity in gene content between the sex chromosomes could be coupled with selective pressure favoring the evolution of mechanisms of dosage compensation. For this reason, the proposal that degenerate sex chromosomes deter polyploidization (including species that lack dosage compensation) is not independent of Orr's (1990) proposal that dosage compensation deters polyploidization. In either case, if sex chromosome degeneration acts as a barrier to polyploidization, this would predict that polyploid species or species with polymorphism in ploidy levels would have relatively "young", minimally degenerate sex chromosomes as compared with other species.

In this chapter we briefly review polyploidization in frogs and salamanders and general features of sex chromosome evolution. Using previously published information, we then use a maximum likelihood approach to analyze the evolution of new sex-determining mechanisms in frogs and salamanders in a phylogenetic context, where new mechanisms are inferred either from a change in which chromosomes are the sex chromosomes, the evolution of a new trigger for sex determination, or from observed polymorphism in sex-determining mechanisms. Following this, we explore whether polyploidization occurs more frequently soon after a new sex-determining mechanism evolves using a permutation test that accommodates uncertainty in ancestral reconstruction. We conclude that novel sex-determining mechanisms have evolved in amphibians even more frequently than previously proposed, and that amphibians with young sex chromosomes may be more likely to experience genome duplication, resulting either in polyploid speciation or in spontaneous polyploidy of individuals of an otherwise diploid species. A major caveat to the latter result is that information on the age of sex-determining mechanisms of most polyploid amphibians is lacking.

18.1.1 Sex Chromosome Evolution

Sex chromosomes originate from autosomes (Ohno 1967) but differ in carrying genetic information that (a) differs between the sexes and (b) triggers or represses sex-specific gonadal differentiation. The "heterogametic" sex produces two types of gametes, each type with a different sex chromosome, and the "homogametic" sex produces only one type of gamete with respect to the sex chromosomes. The sex chromosomes of species with male heterogamy are called "X" and "Y" (females

have two X chromosomes and males have an X and a Y), and the sex chromosomes of species with female heterogamy are called "Z" and "W" (males have two Z chromosomes, and females have a Z and a W). In species with genetic sex determination, gonadal differentiation—also known as primary sex determination—is achieved either using a sex chromosome-specific genetic trigger or by gene dosage, where the homogametic sex carries two doses of an activator of that sex, or a repressor of the heterogametic sex.

The age of sex chromosomes influences important aspects of their evolution and divergence, including divergence in gene content, and the origin of dosage compensation. For example, the sex chromosomes of therian (placental and marsupial) mammals are extremely old (>180 million years; Graves 2008), and the Y chromosome is much smaller than the X and carries fewer genes. This disparity in size and gene content arose due to Y chromosome "degeneration" as a consequence of suppressed recombination with the X chromosome (Charlesworth and Charlesworth 2000). Suppressed recombination ensures that male progeny inherit an intact copy of the genetic trigger for testis formation, which in therians is the *SRY* gene. But this also permits deleterious mutations to accumulate in Y-linked genes (Muller 1964), leading to loss of function and deletion. "Muller's ratchet", the stochastic loss of the least deleterious allele in a population (Felsenstein 1974), leads to a decline in fitness. This decline occurs more quickly in regions of the genome that do not recombine. Hill-Robertson effects, background selection, and hitchhiking of deleterious alleles also contribute to fitness declines of non-recombining portions of the genome (reviewed in Charlesworth and Charlesworth 2000). Degeneration of the therian Y-chromosome occurred in a stepwise fashion as the region of suppressed recombination expanded in large increments (Skaletsky et al. 2003). In therians the disparity in gene content increased over time after the origin of SRY and associated suppression of recombination between the X and Y chromosomes. Most angiosperm plants that have separate sexes (dioecy), in contrast, have comparatively young sex chromosomes that are not substantially differentiated, although exceptions exist (Bergero and Charlesworth 2011; Charlesworth 2002). Polyploid species are prevalent in angiosperms (Otto and Whitton 2000) but absent in therians (Svartman et al. 2005), and these observations thus provide anecdotal support for the contention that the extent of sex chromosome degeneration is negatively correlated with the incidence of polyploid speciation. Amphibians offer an interesting focal group with which to further evaluate this hypothesis because some features of amphibian genome evolution resemble plants more than other animal groups such as therian mammals. In particular, unlike therian mammals, sex chromosomes in many amphibians are relatively young, chromosome degeneration is modest or absent, and polyploidization is fairly common.

Species that determine sex exclusively using environmental triggers do not have genomic differences between the sexes and therefore have no sex chromosomes. In amphibians, sex determination is genetic so all species are expected to have sex chromosomes. In addition, temperature has been reported to influence offspring sex ratios of various species of the salamander genera *Pleurodeles* and

Hynobius and the frog genera *Bufo*, *Rana*, and laboratory-generated polyploids of the genus *Xenopus* (Hayes 1998; Kobel 1996; Schmid and Steinlein 2001). Sex chromosomes are cytologically distinct in some amphibian species (Schmid et al. 2010). Differences in gene content between the sex chromosomes, which is suggested by cytologically distinct sex chromosomes, led to the independent evolution of dosage compensation mechanisms in placental mammals, birds, and other groups such as *Drosophila* and *Caenorhabditis* (Arnold et al. 2008; Straub and Becker 2007). However, in amphibians evidence of dosage compensation has not been found (Hayes 1998; Schmid et al. 1986; Schmid and Steinlein 2001). One possible reason for this is that we do not yet know the identity of any amphibian genes that are hemizygous in the heterogametic sex, so a rigorous test for dosage compensation in amphibians is not yet possible. These genes would be restricted to the portion of the X or Z chromosome that does not recombine with the Y or W chromosome, respectively.

A recent study of European tree frogs identified one way that amphibians circumvent sex chromosome degeneration (Stöck et al. 2011). In three species, no recombination occurred between the sex chromosomes in males generated from intraspecific crosses, yet no intraspecific sex chromosome divergence was observed (Stöck et al. 2011). This suggests that Muller's ratchet is periodically reset in these species by infrequent recombination between the sex chromosomes. Sex chromosome degeneration can also be circumvented by genomic translocation of the sex-determining locus to another chromosomal pair, or by re-assignment of the sex-determining function to a gene located elsewhere in the genome. Both of these phenomena result in a change in which chromosomes are the sex chromosomes (hereafter "sex chromosome turnover"). Ancient examples of sex chromosome turnover are evinced in amniotes by homology between the sex chromosomes of platypuses and those of birds but not those of therian mammals (Graves 2008). In amphibians, sex chromosome turnover is common and is suggested by variation among and within species in male versus female heterogamy (Ezaz et al. 2006; Hillis and Green 1990). Using maximum parsimony, Hillis and Green (1990) analyzed variation in male and female heterogamy in amphibians in a phylogenetic context and concluded that sex chromosome turnover occurred at least seven times.

18.1.2 How Many Frog and Salamander Species are Polyploid?

Comprehensive reviews of polyploidization in amphibians are available in Bogart (1980), Kawamura (1984), Duellman and Trueb (1994), Beçak and Beçak (1998), Otto and Whitton (2000), Gregory and Mable (2005), Schmid et al. (2010), and Mable et al. (2011). The two most recent of these reviews have up-to-date lists of known polyploid species and associated citations that document polyploidy. Schmid et al. (2010) also summarize male and female heterogamy in frogs and salamanders, including information on species with cytologically detectable sex

chromosome divergence (their Table 8, pp 160–161). In their Supplementary Information, Mable et al. (2011) provide data on confirmed diploid species that are closely related to the polyploids. A key difference between these two reviews is that Mable et al. (2011) include only bisexually reproducing polyploids whereas Schmid et al. (2010) also include unisexual polyploids. We have attempted to compile this information as inclusively as possible in Table 18.1, including some minor corrections and a few additional species and associated citations. Thus, not all polyploids listed in this table are bisexual, and some are diploid species in which polyploid individuals occur spontaneously or by induction due to laboratory manipulation.

Fifty polyploid frog species have been described, including seven triploids, 30 tetraploids, 11 octoploids, and two dodecaploids derived from 15 families and 20 genera (Table 18.1). Three tetraploids and two dodecaploids have been reported from the genus *Xenopus* but not yet formally described as species (Evans 2007, 2008; Evans et al. 2004a, 2005a; Tymowska 1991). Stable triploids are known from three frog genera (*Bufo*, *Eupsophus*, and *Rana*), tetraploids from 16 (*Aphantophryne*, *Astylosternus*, *Bufo*, *Chiasmocleis*, *Dicroglossus*, *Eleuthrodactylus*, *Hyla*, *Neobatrachus*, *Odontophrynus*, *Phyllomedusa*, *Pleurodema*, *Pyxicephalus*, *Scaphiophryne Silurana*, *Tomopterna*, and *Xenopus*), octoploids from three (*Ceratophrys*, *Pleurodema*, and *Xenopus*), and dodecaploids only from *Xenopus*. Spontaneous or experimentally induced polyploidy has been reported in at least five frog species. Six polyploid species of salamander, including four triploids and two tetraploids, are known from only two genera (*Ambystoma* and *Siren*) from two families (Table 18.1). Spontaneous or experimentally induced triploidy or tetraploidy has been reported in eight salamander species.

The origin of polyploidy necessarily is preceded by the existence of one or more diploid ancestors. Interestingly, a number of polyploid frog species are inferred to have originated from ancestral diploid species that do not have known extant diploid descendants. In *Xenopus* and *Silurana*, for example, three currently unknown diploid species contributed their genomes to extant tetraploid species (reviewed in Evans 2008). There are also three currently unknown tetraploid species that contributed their genomes to extant octoploid and dodecaploid *Xenopus* species (reviewed in Evans 2008). The tetraploid *Hyla versicolor* is thought to be derived from multiple independent allopolyploidization events between three diploid species, two of which are currently unknown, and probably extinct given that the region in which they occur (temperate North America) is well studied (Holloway et al. 2006). In *Ceratophrys*, there are no known tetraploid species even though three species in this genus are octoploid (Table 18.1). Similarly, the tetraploid species *Bufo pewzowi* is thought to be derived from the diploid *B. turanensis* and another unidentified diploid (Stöck et al. 2009), and various tetraploid species of *Neobatrachus* are derived from diploid ancestors whose diploid descendants are currently unknown (Mable and Roberts 1997). It is tempting to speculate from these observations that polyploidization contributed to the long-term survival of these lineages, given that the diploid ancestors of extant polyploids seem to have gone extinct in many cases. However, we lack information on how frequently

Table 18.1 A list of known polyploid amphibians compiled primarily from Schmid et al. (2010) and Mabel et al. (2011). Additional citations are provided for examples not included in these references and for unnamed species

Family and species (Frogs)	Ploidy	Family and species (Frogs continued)	Ploidy
Arthroleptidae		Microhylidae	
Astylosternus diadematus	Tetraploid	*Aphantophryne (Cophixalus) pansa*	Tetraploid
		Chiasmocleis leucosticta	Tetraploid
Bufonidae[1]			
Bufo poweri	Triploid	Pipidae	
Bufo baturae	Triploid	*Silurana epitropicalis*	Tetraploid
Bufo pseudoraddei[1]	Triploid	"*Silurana* new tetraploid 1" ("*Silurana* sp. Nov VII", "Silurana paratropicalis")[2,3]	Tetraploid
Bufo zugmayeri[1]	Triploid	"*Silurana* new tetraploid 2"[2]	Tetraploid
Bufo viridis	Triploid	*Xenopus borealis*	Tetraploid
Bufo kerinyagae	Tetraploid	*Xenopus clivii*	Tetraploid
Bufo asmaerae/ Amietophrynus asmaerae	Tetraploid	*Xenopus fraseri*	Tetraploid
Bufo oblongus and subspecies (synonym of "*B. danatensis*")	Tetraploid	*Xenopus gilli*	Tetraploid
Bufo pewzowi and subspecies (also a synonym of *"B. danatensis"*)	Tetraploid	*Xenopus laevis*[4]	Tetraploid
		Xenopus muelleri	Tetraploid
Ceratophryidae		*Xenopus pygmaeus*	Tetraploid
Ceratophrys dorsata/ Ceratophrys aurita	Octoploid	*Xenopus largeni* ("*Xenopus* sp. Nov. III")[5]	Tetraploid
Ceratophrys ornata	Octoploid	"*Xenopus* new tetraploid 1" *("Xenopus* sp. Nov. VI"," *Xenopus* muelleri west")[5,6]	Tetraploid
Ceratophrys joazeirensis	Octoploid	*Xenopus amieti*	Octoploid
		Xenopus andrei	Octoploid
Craugastoridae		*Xenopus boumbaensis*	Octoploid
Eleutherodactylus (Haddadus) binotatus	Tetraploid	*Xenopus itombwensis*[7]	Octoploid
		Xenopus lenduensis[8]	Octoploid
Cycloramphidae		*Xenopus vestitus*	Octoploid
Eupsophus vertebralis	Triploid	*Xenopus wittei*	Octoploid

(continued)

Table 18.1 (continued)

Family and species (Frogs)	Ploidy	Family and species (Frogs continued)	Ploidy
Odontophrynus americanus	Tetraploid	*"Xenopus* sp. nov. X"[5,9]	Octoploid
		Xenopus longipes	Dodecaploid
Dicroglossidae		*Xenopus ruwenzoriensis*	Dodecaploid
Dicroglossus (Hoplobotrachus) occipitalis	Tetraploid	*"X. cf. boumbaensis"*[10]	Dodecaploid
		"Xenopus sp. Nov. VIII"[5,9]	Dodecaploid
Hylidae			
Hyla versicolor	Tetraploid	Pyxicephalidae	
Phyllomedusa tetraploidea	Tetraploid	*Pyxicephalus (Tomopterna) delalandii*	Tetraploid
Leiuperidae		Ranidae	
Pleurodema bibroni	Tetraploid	*Rana esculenta*	Triploid
Pleurodema cordobae	Octoploid	*Rana japonica**[11]	Triploid
Pleurodema kriegi	Tetraploid	*Rana nigromaculata***	Triploid/ Tetraploid
		*Rana pipiens**[12]	Triploid
Leiopelmatidae		*Rana rugosa**[13]	Triploid
*Leiopelma hochstetteri***	Triploid	*Tomopterna tandyi*	Tetraploid
Limnodynastidae		Scaphiophidae	
Neobatrachus aquilonius	Tetraploid	*Scaphiophryne gottlebei*	Tetraploid
Neobatrachus centralis	Tetraploid		
Neobatrachus kunapalari	Tetraploid		
Neobatrachus sudelli	Tetraploid		

Family and species (Salamanders)	Ploidy
Ambystomatidae	
Ambystoma jeffersonianum	Triploid
*Ambystoma mexicanum***	Triploid/Tetraploid
Ambystoma nothagenes	Triploid
Ambystoma platineum	Triploid
Ambystoma tremblayi	Triploid
Plethodontidae	
*Eurycea bislineata***	Triploid/Tetraploid
Salamandridae	
*Notophthalmus viridescens**	
*Pleurodeles waltl**	Triploid
*Triturus alpestris**	Triploid
*Triturus pyrrhogaster***[14]	Triploid
*Triturus viridescens***[15]	Triploid
*Lissotriton vulgaris***[16]	Triploid
Sirenidae[17]	
Siren intermedia	Tetraploid
Siren lacertina	Tetraploid

(continued)

Table 18.1 (continued)

Family and species (Salamanders)	Ploidy

* Experimentally induced

** Spontaneously observed, in some cases also experimentally induced

[1] Following taxonomy of Stöck M, Ustinova J, Lamatsch DK, *et al.* (2009) A vertebrate reproductive system involving three ploidy levels: Hybrid origin of triploids in a contact zone of diploid and tetraploid paleartic green toads (*Bufo viridis* subgroup). *Evolution* **64**, 944–959. Further work is needed to confirm stable triploid ploidy of *B. pseudoraddei* and *B. zugmayeri* (Stöck et al. 2009)

[2] Evans BJ, Kelley DB, Tinsley RC, Melnick DJ, Cannatella DC (2004) A mitochondrial DNA phylogeny of clawed frogs: phylogeography on sub-Saharan Africa and implications for polyploid evolution. Molecular Phylogenetics and Evolution 33, 197–213

[3] "Silurana paratropicalis" is a nomen nudem; see Blackburn DC (2011)

[4] Here we consider as *X. laevis* all diverged populations within this clade as identified by Evans et al. (2004). This includes *Xenopus sp. Nov. IX (a.k.a.* X. congo 3) from Tymowska (1991), *X. petersi*, and *X. victorianus*

[5] Tymowska J (1991) Polyploidy and cytogenetic variation in frogs of the genus Xenopus. In: Amphibian cytogenetics and evolution (eds. Green DS, Sessions SK), pp. 259–297. Academic Press., San Diego

[6] Kobel HR, Loumont C, Tinsley RC (1996) The extant species. In: The Biology of *Xenopus* (eds. Tinsley RC, Kobel HR), pp. 9–33. Clarendon Press, Oxford

[7] Table 13 of Schmid et al. (2010) incorrectly lists *Xenopus itombwensis* as a dodecaploid

[8] Evans BJ, Greenbaum E, Kusamba C, et al. (2011) Description of a new octoploid frog species (Anura: Pipidae: Xenopus) from the Democratic Republic of the Congo, with a discussion of the biogeography of African clawed frogs in the Albertine Rift. Journal of Zoology, London 283, 276–290

[9] Species status requires further investigation; for example, *Xenopus* sp Nov. VIII may be the same as *Xenopus cf. boumbaensis*

[10] Evans BJ (2007) Ancestry influences the fate of duplicated genes millions of years after duplication in allopolyploid clawed frogs (Xenopus). Genetics 176, 1119–1130

[11] Kawamura T, Tokunaga C (1952) The sex of triploid frogs, *Rana japonica* Günther. Journal of Science of the Hiroshima University, Series B, Division 1 (Zoology) 13

[12] Briggs R (1947) The experimental production and development of triploid frog embryos. Journal of Experimental Zoology 106, 237–266

[13] Kashiwagi, K. (1993) Production of triploids and their reproductive capacity in *Rana rugosa*. Sci. Rep. Lab. Amphibian Biol. Hiroshima Univ. 12: 23–36

[14] Fankhauser, G, Crotta, R., Perrot, M. (1942) Spontaneous and cold-induced triploidy in the japanese newt, Triturus pyrrhogaster. Journal of Experimental Zoology 89(1) 167-181

[15] Fankhauser, G. (1941) The frequency of polyploidy and other spontaneous aberrations of chromosome number among larvae of the newt, *Triturus viridescens*. PNAS 27(11): 507-512. Fankhauser, G. and Watson, R. C. (1942) Heat-indiced triploidy in the Newt, *Triturus viridescens*. Proceedings of the National Academy of Sciences 28(10): 436–440

[16] Litvinchuk, S. N., Rosanov, J. M., Borkin, L. J. 1998. A case of natural triploidy in a smooth newt Triturus vulgaris (Linneaus, 1958), from Russia (Caudata: Salamandridae). Herpetozoa 11: 93-95

[17] Morescalchi A, Olmo E (1974) Sirenids: a family of polyploid Urodeles? Experientia 30, 491–492 found *Pseudobranchus striatus* to be polyploid but this result was not supported by the analysis of Moler PE, Kezer J (1993) Karyology and systematics of the salamander genus Pseudobranchus (Sirenidae). Copeia 1993, 39–47

polyploidization occurs and how frequently diploids outcompete polyploids, so it is difficult to test this. It is also plausible, for example, that variation in ploidy level is a neutral phenomenon influenced by stochastic survival and extinction of polyploids and diploids, or by variation among lineages, including polyploids (Mayrose et al. 2011), in their ability to speciate by polyploidization.

18.1.3 Examples of polyploidy in species with demonstrably young sex chromosomes

About one third of the described polyploid frog species belong to the genus *Xenopus*. At least six independent instances of genome duplication gave rise to the ploidy levels seen among extant species in this group, including multiple episodes that generated the highest ploidy level of any vertebrate—dodecaploidy (reviewed in Evans 2008). Tetraploid *Xenopus* evolved at least once, octoploid *Xenopus* evolved independently at least three times, and dodecaploid *Xenopus* evolved independently at least two times (and possibly more depending on the species status of *Xenopus* cf. *boumbaensis* and of *Xenopus* sp. nov. VIII; Table 1, Evans 2007, 2008; Evans et al. 2008a, 2011, 2005a; Tymowska 1991). Tetraploidy also occurred independently in *Silurana* (Evans 2007; Evans et al. 2005a).

With respect to genome duplication, something is clearly special about *Xenopus*—but what? One possible clue emerges from the recent discovery of the first known genetic trigger of sex determination in amphibians by Yoshimoto et al. (2008). These researchers identified a female-specific gene called *DMW* in the tetraploid species *Xenopus laevis*. *DMW* is a W-chromosome linked gene that evolved via gene duplication from another important regulator of sexual differentiation called *DMRT1* (Yoshimoto et al. 2008). *DMW* may function by blocking *DMRT1* induction of testis differentiation (Yoshimoto et al. 2010, 2008). Potentially relevant to the high incidence of polyploidization in *Xenopus* is the discovery that *DMW* originated extremely recently in amphibian evolution—after divergence of *Silurana* and *Xenopus*, but before diversification of most or all extant species of *Xenopus* (Bewick et al. 2011). Not surprisingly, the sex chromosomes of *Xenopus* are not cytologically distinct (Tymowska 1991). Gene contents of the W and Z chromosomes of *Xenopus* are therefore probably very similar, and *Xenopus* species presumably lack mechanisms of dosage compensation operating over most sex-linked genes because both sexes have two alleles at most loci on the sex chromosomes. The preponderance of polyploids in *Xenopus* is therefore consistent with the proposal that polyploidization is more likely to occur in lineages with young, minimally degenerate sex chromosomes.

Another possible link between sex chromosome evolution and polyploidization is provided by *Leiopelma hochstetteri*. This species has intraspecific variation in the presence of a recently evolved univalent W chromosome that governs sex determination in females (Green 1988). *Leiopelma hochstetteri* is diploid but also has spontaneous triploidy (that is, polyploidy without speciation;

Green et al. 1984). It is not clear whether novel mechanisms for sex determination are more likely to evolve and persist in species that have nondegenerate sex chromosomes, but this seems plausible under the same reasoning discussed above with respect to the propensity for lineages to experience polyploidization. More specifically, if a new pair of sex chromosomes appears in a population then the old ones would segregate as a newly established autosomal pair. For this reason, ancestral sex chromosomes with similar gene content would lack or have few null alleles when they segregate autosomally. While the observation of spontaneous triploidy suggests a tolerance of polyploidy, *L. hochstetteri* is not a polyploid species, so a direct link between the age of the sex chromosomes and polyploid speciation (as opposed to the toleration of polyploidy) is not established by this species.

18.2 Evolution of Sex Determination Systems in Amphibians

18.2.1 Methods

Changes in the heterogametic sex, evolution of new triggers for sex determination, and polymorphism in sex chromosomes mark the origin of novel features in genetic pathways for sex determination. In order to quantify how many times this has happened in amphibians, we began with the large amphibian phylogeny reported by Pyron and Wiens (2011). We trimmed from this tree all species except those for which we had information on either heterogamy or polyploidy, or both, and retained the original maximum likelihood branch lengths among the retained species. For illustrative purposes, we also retained diploid species (confirmed or presumed) from the phylogeny of Pyron and Wiens (2011) that are closely related to polyploid species. In many cases diploidy has been confirmed in these species or other closely related species (see Supplementary Information of Mable et al. 2011). We had heterogamy information for *Physalaemus (Engystomops) petersi*, but this species was not included in the phylogeny. Therefore, we used a closely related species (*Physalaemus cuvieri*) that is included in the phylogeny to represent *Physalaemus petersi*. *Physalaemus petersi* was the only species from this genus that was analyzed, so this substitution should be uncontroversial (note that placing some *Physalaemus* in *Engystomops* makes no difference as *Engystomops* and *Phsalaemus* are sister taxa). To better illustrate the phylogenetic distribution of polyploid species in Fig. 18.1, we also substituted *Chiasmocleis hudsoni*, which was present in the phylogeny of Pyron and Wiens (2011), with the tetraploid species *C. leucosticta*, which was not present in the phylogeny of Pyron and Wiens (2011). However, *C. leucosticta* was not included in any of the analyses described below because we lack data on heterogamy for this species.

A total of 143 species (97 frogs, 45 salamanders, and one caecilian as an outgroup) were included. We then converted this tree to a chronogram (a time-

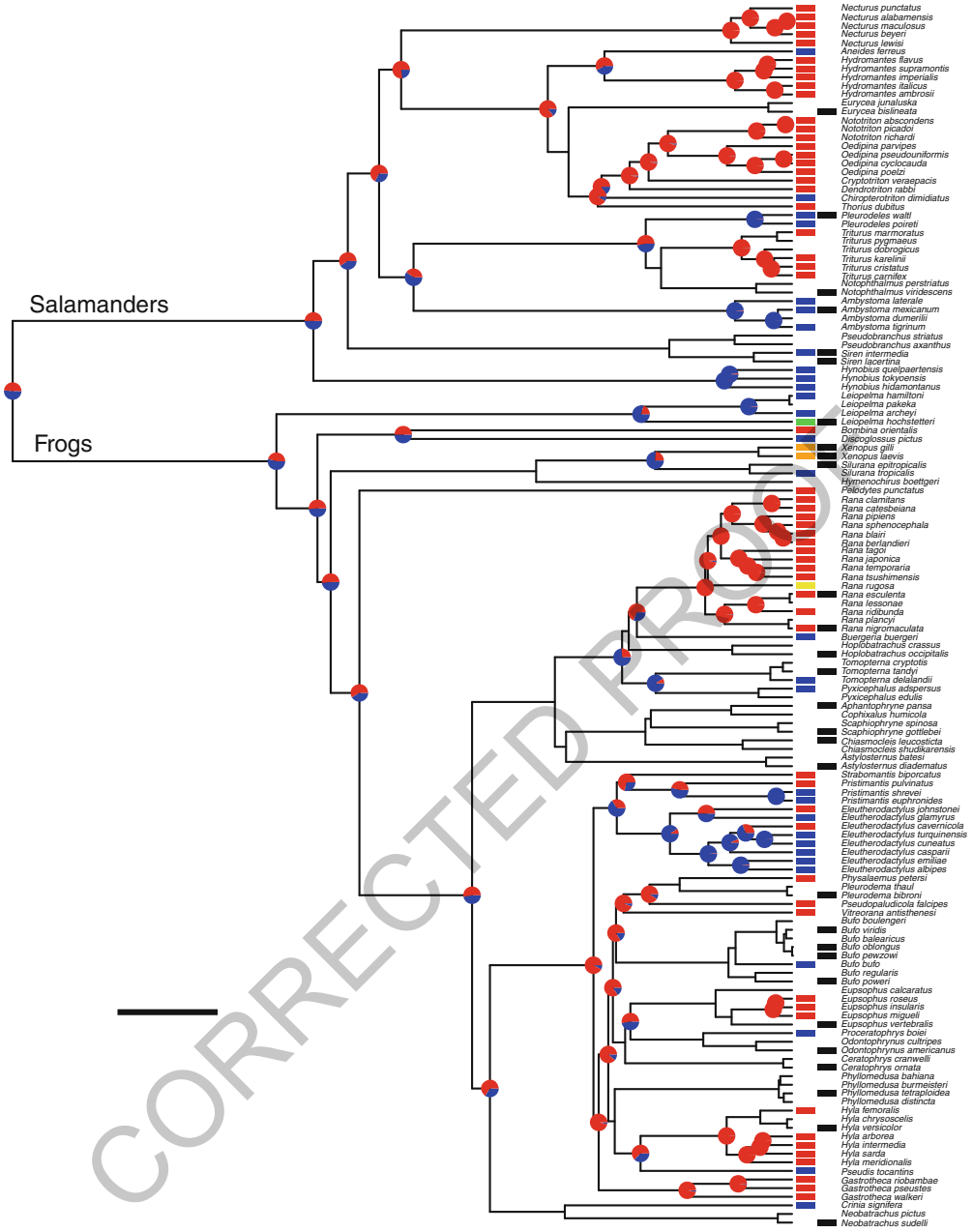

Fig. 18.1 Heterogamy and polyploidy in salamanders and frogs. The phylogeny is from Pyron and Wiens (2011) with branch lengths proportional to time based on a relaxed molecular clock and calibration points described in the text. A *black* scale bar indicates 40 million years of evolution. *Red* and *blue rectangles* on tips indicate male or female heterogamy respectively; *green*, *orange*, and *yellow rectangles* indicate de novo sex determining systems; missing data on heterogamy have no *rectangles* in this column. In the *right* column, *black rectangles* indicate polyploid species; other species are either confirmed or assumed diploid. Some polyploid species listed in Table 18.1 are not included in this figure due to a lack of phylogenetic information. Pie charts on nodes indicate ancestral reconstructions of heterogametic state

calibrated phylogeny) using the penalized likelihood approach (Sanderson 2002), implemented in r8s version 1.71 (Sanderson 2003). We used the calibration points detailed in Wiens (2011) but some had to be excluded given the more limited taxon sampling used here, and some were added or modified given the differences in taxon sampling (e.g., we added two calibration points within the genus *Hyla*, given our more extensive sampling of species in that genus relative to Wiens (2011). We used the following 17 calibration points. The first 16 were treated as constraints on the minimum age of each clade, and the final calibration point was a fixed age for the root of the tree.

(1) Most recent common ancestor (MRCA) of extant salamanders, at least 150.8 Mya (Millions of years ago), based on the fossil *Iridotriton hechti* of the Kimmeridgian/Early Tithonian (Late Jurassic), which is considered to be a crown-group caudate (Evans et al. 2005b).
(2) MRCA of Salamandroidea (all salamanders exclusive of cryptobranchids, hynobiids, and sirenids), at least 125.0 Mya (early Barremian, Cretaceous), based on *Galverpeton* and *Valdotriton* (Evans and Milner 1996).
(3) MRCA of plethodontids and proteids, at least 65.5 Mya. The oldest known amphiumid fossil (*Proamphiuma cretacea*) is late Maastrichtian or early Paleocene, and thus from approximately 65.5 Mya (Gardner 2003). The split between Plethodontidae and Amphiumidae must be at least this old. We do not have amphiumids included here, but the clade of plethodontids and proteids must be at least this old given the well-supported clade consisting of proteids, rhyacotritonids, amphiumids, and plethodontids; see Pyron, Wiens (2011) and earlier studies.
(4) MRCA of *Aneides* and *Hydromantes*, at least 19 Mya. Given the presence of an *Aneides* vertebra in the Arikareean period (Tihen and Wake 1981), the MRCA of the clade containing modern *Aneides* must be at least 19 Myo (Millions of years old).
(5) MRCA of *Triturus* and *Notophthalmus* at least 33.9 Mya, based on fossils of *Triturus* from the Eocene of Europe (33.9–55.8 Mya; Milner 2000)
(6) MRCA of Ambystomatidae and Salamandridae, at least 56.8 Mya, based on a fossil dicamptodontid (Paleocene; Tiffanian; 60.2–56.8 Mya; Naylor and Fox 1993), and given that the ambystomatidae is the sister group to the Dicamptodontidae (so the sister group to Ambystomatidae + Dicamptodontidae must be at least this old).
(7) MRCA of frogs and salamanders, at least 245 Mya, based on a fossil anuran (*Triadobatrachus*) from the Early Triassic (251–245 Mya) of Madagascar (Carroll 1988; Rage and Rocek 1989)
(8) MRCA of pipoids and all other frogs, at least 145.5 Mya, given *Rhadinosteus parvus*, ostensibly a rhinophrynid and clearly a pipoid, from the Late Jurassic (Tithonian, 145.5–150.8 Mya; Rocek 2000).
(9) MRCA of *Hymenochirus* and *Xenopus*, at least 83.5 Mya, given the pipid *Pachybatrachus taqueti* from the Upper Cretaceous (Coniacian-Santonian, 83.5–89.3 Mya), which is thought to be closely related to *Hymenochirus* (Rocek 2000).

(10) MRCA of Myobatrachidae (represented here by the limnodynastine *Neobatrachus* and the myobatrachine *Crinia*) at least 54.6 Mya, given fossils assigned to the limnodynastine genus *Lechriodus* (Evans et al., 2008b; Sanchiz, 1998).

(11) MRCA of Bufonidae + Leptodactylidae + Centrolenidae, at least 55.8 Mya, given putative fossil *Bufo* from the late Paleocene (55.8–58.7 Mya; Baéz 2000).

(12) MRCA of Ranidae (sensu Wiens et al. 2009) at least 33.9 Mya, given fossil *Rana* from the Late Eocene (37.2–33.9 Mya; Rocek and Rage 2000).

(13) Crown group of Terrarana (the clade including the families Brachycephalidae, Ceuthomantidae, Craugastoridae, Eleutherodactylidae, and Strabomantidae, or more simply, the clade including *Eleutherodactylus* and related genera) at least 35 Mya, based on an *Eleutherodactylus* fossil in amber from the La Toca formation (Dominican Republic) estimated to be ~35 Myo (Poinar and Cannatella 1987).

(14) Stem group of Ceratophryidae (the clade including the genera *Ceratophrys*, *Chacophrys*, and *Lepidobatrachus*) at least 65.5 Mya based on the late Cretaceous fossil genera *Beelzebufo* and *Baurubatrachus* (Evans et al. 2008b). Evans et al. (2008b) considered the Madagascan taxon *Beelzebufo* to be a ceratophryine. This taxon is of Maastrichtian (Late Cretaceous) age (65.5–70.6 Mya). The South American genus *Baurubatrachus* is also considered to be a ceratophryine (Evans et al. 2008b; Rocek 2000). Although the exact relationships of these taxa are somewhat uncertain, the presence of seemingly ceratophryine fossils in South America suggests that the stem group age of Ceratophryidae is at least 65.5 Mya. The relationships of ceratophryids are uncertain, but in this molecular analysis, they appear as the sister group to a clade including Odontophrynidae and Alsodidae (*Eupsophus)*.

(15) Crown-group age of North American and European *Hyla* clade, at least 16 Myo; given fossil *Hyla* similar to extant *H. arborea* and *H. meridionalis* in the Lower Miocene of Austria (~16 Myo; Sanchiz 1998). We assume that these *Hyla* are closely related to *Hyla* presently extant in Europe. However, we cannot assume that these fossils are younger than the crown-group age of the extant European species. We assume instead that the crown group of the clade of *Hyla* is at least 16 Myo based on these European fossils.

(16) MRCA of *H. avivoca-H. chrysocelis-H. versicolor* clade; *H. miocenica* is thought to be closely related to *H. chrysocelis* and *H. versicolor* and occurs in the Barstovian of the Middle Miocene (14–16 Myo; Holman 2003). In our phylogeny, *H. avivoca, H. chrysocelis*, and *H. versicolor* form a clade. We assume that the stem group age of these three species is at least 14 Myo.

(17) We fixed the root age of the tree using the estimated age from Wiens (2011) for the MRCA of lissamphibians (frogs, salamanders, caecilians) of 368.3 Mya, using penalized likelihood. Although the use of a fixed calibration point (rather than a minimum constraint) may seem controversial, it should be noted that at least one node must be given a fixed age. Furthermore, our focus here is not on re-estimating these ages, but providing relative assessments of clade ages (see below).

Construction of a chronogram using r8s requires a cross-validation step that identifies a best-fitting value for the "smoothing parameter", which specifies the cost of differing rates of evolution between neighboring branches (Sanderson 2002). Cross-validation considered smoothing parameter values from 10^0 –$10^{5.5}$ in exponential increments of 0.5. These cross-validation analyses failed until a species with a zero-length branch (*Bufo pewzoi*) was removed. After removing this species, the cross-validation analyses showed that a value of 10^1 gave the lowest Chi-squared error. We then generated a chronogram for the 142 remaining species using this smoothing parameter. We then performed a second analysis using this same smoothing parameter but including all 143 species. This second analysis gave identical divergence-date estimates throughout the tree as the first analysis with 142 species. The resulting chronogram (Fig. 18.1) shows species for which we have information on phylogenetic relationships and either heterogamy and/or ploidy.

Using the R package "ape" (Paradis et al. 2004), which is a software package for phylogenetic analysis, we tested the fit of alternative models for the evolution of new sex determination systems for a total of 90 species (55 frogs and 35 salamanders) for which we had data on heterogamy or sex chromosome polymorphism. This was done to select an appropriate model for ancestral reconstruction and for use in a permutation test described below. We coded all species as having either male heterogamy (0), female heterogamy (1), or, for each of three species (*Xenopus laevis*, *Leiopelma hochstetteri*, and *Rana rugosa*), a unique "de novo" sex-determining mechanism (2, 3, and 4), in which categories we include species with polymorphisms in mechanisms for sex determination. *Xenopus laevis* was assigned a unique heterogametic state in order to account for the finding that this species evolved its W-linked sex-determining gene after the split from *S. tropicalis* (Bewick et al. 2011), which also has female heterogamy. *Leiopelma hochstetteri* was assigned a unique heterogametic state in order to account for the finding that this species recently evolved a derived W0 sex-determining system that is unique to this lineage (Green et al. 1993; Sharbel et al. 1998). *Rana rugosa* was assigned a unique heterogamy state in order to accommodate evidence for recent and possibly repeated instances of sex chromosome turnover (Ogata et al. 2008). The three de novo states were coded as separate character states in order to ensure that known instances of novel sex determination mechanisms were included in the analysis even though they did not necessarily involve a change in heterogamy. We note that the newly evolved sex chromosomes of *L. hochstetteri* and *R. rugosa* are polymorphisms, and it is not clear whether these new polymorphisms will eventually fix in each species, and thus actually constitute a sex chromosome turnover. However, in both of these examples, at least one of the polymorphic systems for sex determination is species-specific and therefore new.

Using the "ace" function of the "ape" package, we then evaluated the following models for evolution of the five heterogamy states:

(1) All rates equal (one rate for all possible transitions between states, one parameter).
(2) One reversible rate between XY and ZW, and one reversible rate to and from any of the de novo states (two rates in total, two parameters). By reversible, we

mean, for example, that the rate of change from XY to ZW is equal to the rate of change from ZW to XY.
(3) One rate for XY to ZW, another rate for ZW to XY, and one reversible rate to and from any of the de novo states (three rates in total, three parameters).
(4) Rates between each of the five heterogamy states are reversible and unique (ten rates in total, ten parameters).
(5) All rates unique (twenty rates in total, twenty parameters).

18.2.2 Results and Discussion

The likelihoods of each of these models were compared using the Akaike Information Criterion (Akaike 1974) calculated as $2k–2\ln(L)$ where k is the number of parameters in the model and L is the maximum value of the likelihood function of the model. A P value was generated with a hierarchical likelihood ratio test (hLRT) with degrees of freedom equal to the difference in free parameters of the models under the assumption of a Chi-squared distribution. For the hLRT we evaluated whether adding complexity to the models resulted in a significant increase in model fit. The likelihoods of Models 1, 2, 3, 4 and 5 were -70.74572 (AIC = 143.5), -53.82688 (AIC = 111.7), -53.81533 (AIC = 113.6), -52.31287 (AIC = 124.6), and -49.97701 (AIC = 140.0) respectively. Model 2 thus was favored by the Akaike Information Criterion. According to the hLRT, Model 2 was also preferred over Model 1 ($P<0.0001$), but Model 3 was not preferred over Model 2 ($P = 0.879$). Model 4 was not preferred over Model 2 ($P = 0.932$) or Model 3 ($P = 0.885$), and Model 5 was not preferred over Model 2 ($P = 0.982$), or Model 3 ($P = 0.999$), or Model 4 ($P = 0.912$). These results suggests that the transition rate from ZW to XY is not significantly higher than the transition rate from XY to ZW. Model 2 was therefore used to reconstruct ancestral heterogamy states and also used for simulations in our permutation test described below. The model used in the ancestral reconstructions differs slightly from the model used in the permutation test in that the rate of reversal from the de novo heterogamy states to other heterogamy states was set to zero for the ancestral reconstructions. This was not possible with the permutation test, which requires a reversible model. We present results from this slightly different version for the ancestral reconstructions for illustrative purposes because with this model there is zero likelihood for all of the de novo states in all of the ancestral reconstructions. Other inferences, such as the likelihood of male and female heterogamy in the most recent common ancestor of frogs, of salamanders, and of frogs and salamanders discussed below, are identical with both of these models.

The ancestral state reconstructions estimated from the analysis with 90 species but plotted on the chronogram with 143 species (Fig. 18.1) suggest that there is not strong statistical support to distinguish whether the ancestral hetrogamy state was female or male heterogamy (that is, ZW females and ZZ males) in frogs or salamanders. The marginal likelihood of female heterogamy (ZW) for the most recent common ancestor of frogs is 0.544, for the most recent common ancestor of

salamanders is 0.499, and for the most recent common ancestor of frogs and salamanders is 0.513. Thus, the support for ZW versus XY heterogamy as the ancestral state in each group is effectively equivocal.

18.3 Is Polyploidy Tolerated to a Greater Degree in Species with Young Sex Chromosomes?

18.3.1 Methods

In our analysis, we consider the sex-determining system to have changed every time that a change in heterogamy occurred from XY to ZW, from ZW to XY, or from ZW or XY to one of the three de novo sex-determining systems. To quantify the number of times that the system for sex determination changed in amphibians, we used the stochastic character mapping approach proposed by Nielsen (2002) as implemented by the R package "phytools" (Revell 2011). This approach simulates character evolution on a phylogeny, conditioning on the observed character states of the terminals. In this way, one can estimate the number of character-state transitions that occurred and also evaluate where in the phylogeny changes are likely to have occurred. The stochastic mapping simulations were performed using a reversible version of Model 2 described above.

As discussed earlier, recent discoveries implicate sex chromosome turnover in facilitating polyploid speciation or the tolerance of polyploidization, at least in *Xenopus* and *Leiopelma*. These observations raise the question: is this a general phenomenon in amphibians? Since a change in heterogamy necessarily involves a change in the sex-determining system, we predicted that the time since a change in heterogamy (XY to ZW or ZW to XY) would be lower in species that are polyploid or that tolerate polyploidy than expected by chance, if the same number of polyploid species (or polyploid-tolerant species) were to evolve randomly on the phylogeny. We note that this hypothesis does not involve correlation between polyploidy and a particular heterogamy state, so standard approaches to test for phylogenetic correlation among traits cannot be used to test this prediction. Instead, we developed a novel permutation test that accommodates uncertainty in when and in which lineages sex chromosome turnover occurred during amphibian evolution.

From the set of 90 species for which heterogamy information was available, we identified five phylogenetically independent instances of stable or spontaneous polyploidy. We emphasize that this is an underestimate of the number of independent polyplodization events, and we were able to use only five instances of polyploidization because we lack heterogamy and/or phylogenetic information for the other examples listed in Table 18.1. Diploidy has either been confirmed for the other species for which heterogamy information was available (Mable et al. 2011) or was assumed. The five examples of independent polyploidization or tolerance of polyploidization are:

(1) *Siren intermedia*, a tetraploid, which may have descended from a tetraploid ancestor that also gave rise to *S. lacertina* (Morescalchi and Olmo 1974). We note that the polyploid status of the family Sirenidae has not been confirmed by additional studies (Mable et al. 2011).
(2) *Ambystoma mexicanum*, a species with spontaneous triploidy (Humphrey 1963) that is closely related to the unisexual triploids *A. jeffersonianum, A. platineum,* and *A. tremblayi* (see notes on species status of unisexuals in Table 18.1).
(3) *Xenopus laevis*, a tetraploid species (Tymowska 1991). We excluded *X. gilli* from this analysis even though we have heterogamy data for this species because it shares a polyploid ancestor with *X. laevis*.
(4) *Rana esculenta,* a naturally occurring diploid/triploid hybridogenic species formed from hybridization of *R. lessonae* and *R. ridibunda* (Uzzell et al. 1975).
(5) *Leiopelma hochstetteri*, a diploid species with spontaneous triploidy (Green et al. 1984).

For this permutation test, we used as a test statistic the mean time since the origin of the current heterogamy state for the five polyploid lineages. This mean was calculated from 1,000 simulations that are conditioned on the observed heterogamy states, using the stochastic character mapping approach described by Nielsen (2002) and implemented by the R package "phytools" (Revell 2011). Each stochastic mapping simulation provides one possible evolutionary scenario that is consistent with the data. It was necessary to perform many (1,000) stochastic mapping simulations for the observed data in order to accommodate uncertainty in these evolutionary scenarios (that is, to accommodate uncertainty in the ancestral reconstruction of the evolution of heterogamy).

If polyploidy tends to occur soon after a change in heterogamy, then the observed test statistic should be smaller than the distribution of statistics calculated after repeatedly randomly selecting five species across the tree to be polyploid, and performing 1,000 stochastic character mapping simulations for each of the randomizations. The observed test statistic was therefore compared to a distribution of statistics generated from 100 randomizations where, in each randomization, five species are selected to be polyploid, with 1,000 stochastic character mapping simulations that were conditioned on the observed heterogamy states performed for each of the randomizations. The difference between the test statistic and the randomizations therefore is that the mean path length (that is, for each polyploid, the path length between the most recent change in heterogamy and the branch tip) was calculated respectively either from real polyploid species (for the test statistic), or from five species selected at random from the 90 species for which we have heterogamy data (for each of the randomizations).

This test is conservative in the sense that it does not consider subsequent, phylogenetically independent instances of polyploidization that occurred in *Xenopus* (three additional independent instances of octoploidization and at least two additional independent instances of dodecaploidization). More specifically, because *Xenopus* has a relatively young sex-determining system, a test statistic generated by counting each of the

independent polyploidization events in *Xenopus* is even lower than the test statistic that counts polyploidization of *Xenopus* only once (see below).

One limitation of this analysis is that the frequency of transitions in amphibian sex-determining systems is undoubtedly underestimated. It is possible, for example, that changes in heterogamy occurred in other species that were excluded in the analysis because differences between the sex chromosomes were not cytologically detectable. It is also possible that some species experienced a change in the sex-determining system that did not involve a change in heterogamy. *Gastrotheca pseustes*, for instance, is known to have polymorphism in the morphology of the Y chromosome (Schmid et al. 1990), but this species was coded as XY because these size variants may involve homologous chromosomes and no change in the sex-determining system. Intraspecific polymorphism in sex-determining mechanisms has also been observed in *Rana narina*, *Eleutherodactylus maussi*, *Rana japonica* and *R. narina* (Eggert 2005), but phylogenetic information was lacking from these species in the phylogeny of Pyron and Wiens (2011). Furthermore, adding more taxa might influence the inferred timing of the transitions to heterogamy, even if the phylogeny remains the same (e.g., added taxa could subdivide long branches and help clarify where on a given branch the heterogamy transition occurred).

Another limitation of this analysis is that we use the total time that the polyploid species have been in the observed heterogamy states for our test statistic, rather than the difference between these times and the age of each polyploid species. The latter difference would be a better metric for this test because it focuses on events prior to polyploidization. However, it is difficult to estimate the age of each polyploid (or of a randomly selected species in the permutation) because in some cases the diploid ancestor of the polyploid is unknown or extinct (see above), or other cases because we lack phylogenetic information from the sister taxon. We note that results are contingent on the phylogeny and evolutionary model, and that this analysis does not accommodate uncertainty in divergence times and phylogenetic relationships.

18.3.2 Results and Discussion

Stochastic mapping of heterogamy state, including the independent evolution of three de novo sex chromosomes provides an average estimated number of times that the sex chromosomes turned over of 32 (95% confidence interval: 25–41) based on 1,000 simulations that were conditioned on the observed heterogamy states. This is much higher than the maximum parsimony inference of only seven changes by Hillis and Green (1990). In the example simulation depicted in Fig. 18.2a, for instance, there are 28 changes in heterogamy.

These simulations also suggest that more changes occurred from male heterogamy to female heterogamy than the reverse, even though our model comparison suggested that the rate of change in each direction was not significantly different. Out of 1,000 stochastic mapping simulations, the mean number of changes from

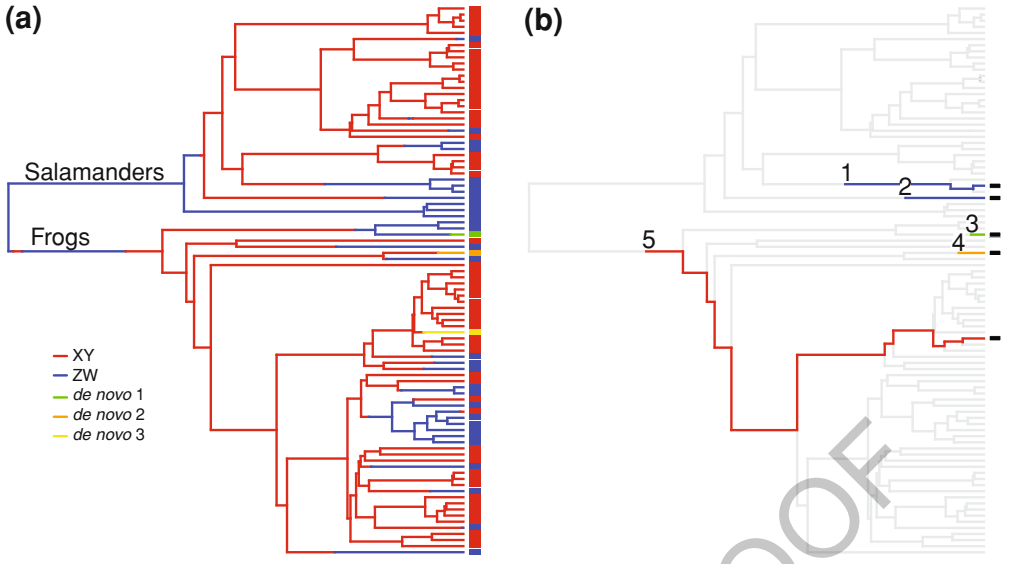

Fig. 18.2 Simulations that stochastically map evolution of heterogamy form the basis of the permutation test. Shown here is (**a**) an example of a stochastic mapping simulation for the evolution of the observed heterogamy states and (**b**) highlighted simulated paths to each of the five observed polyploid species. Species with missing heterogamy data from Fig. 18.1 have been removed for these analyses and species names are omitted for clarity. In (**b**) simulated ages of the observed heterogamy states for *Ambystoma mexicanum*, *Siren intermedia*, *Leiopelma hochstetteri*, *Xenopus laevis*, and *Rana esculentia* are labeled 1, 2, 3, 4, and 5 respectively. The test statistic is the average age of the observed heterogamy state for these five polyploid species, averaged over 1,000 stochastic mapping simulations. This test statistic is compared to analogous calculations from 100 permutations where five species were randomly selected to be polyploid and 1,000 stochastic mapping simulations were performed for each randomization

male to female heterogamy (XY to ZW) was 22 (95 % confidence interval: 13–28) and the mean number of changes from female to male heterogamy (ZW to XY) was 7 (95 % confidence interval: 2–14). The example simulation depicted in Fig. 18.2a is typical of the other simulations in the sense that there are 19 changes from male to female heterogamy but only 6 changes from female to male heterogamy.

The observed average path length to a change in the sex-determining system for the five polyploids averaged over 1,000 stochastic mapping simulations, was 89.0 million years. This is not to suggest that no sex chromosome degeneration occurred within this period. In fact, if sex chromosome degeneration in amphibians occurred at a similar rate as it did in therian mammals (4.6 genes per million years; Graves 2004), the ancestors of these polyploids probably did have somewhat degenerate sex chromosomes. Rather, this result suggests that the amount of sex chromosome degeneration that typically occurs within this period of time was not of sufficient magnitude to prevent polyploidization or the tolerance of spontaneous polyploidization. The permutation test indicates that this test statistic is not significantly lower than the distribution of statistics calculated when polyploids

evolved five times on random branches in this phylogeny ($P = 0.059$; average path length in permutations was 141.2 million years and the standard deviation was 32.7 million years), although the P value is close to 0.05. If we include six independent polyploidizations in *Xenopus* (one tetraploidization, three octoploidizations, and at least two dodecaploidizations; reviewed in Evans 2008) in the observed test statistic, in addition to the four other examples of polyploidization itemized above, the average observed path length to a change in the sex-determining system for the ten independent polyploid lineages is 68.1 million years. This test statistic is significantly smaller than statistics calculated from 100 permutations where one of the five randomly selected polyploids is also assumed to undergo six independent polyploidizations ($P = 0.020$; average path length in permutations was 169.8 million years and the standard deviation was 55.3 million years). Although there are at least six independent polyploidizations in *Xenopus*, this test suffers from pseudoreplication in that these polyploid lineages may share the same system for sex determination (i.e., *DMW*). Additional data on whether other polyploid species of amphibians have male or female heterogamy would clearly help illuminate the question of whether species with young sex chromosomes are more tolerant of polyploidization. It is surprising how little is known about heterogamy of polyploid amphibians given that karyotypes of essentially all of these species were inspected in order to identify polyploidy in the first place. One possible reason for this dearth of information on heterogamy of polyploid species is that many of these species may lack morphologically distinct sex chromosomes. This proposal, if accurate, would be consistent with the contention that polyploidization is better tolerated by species with minimally degenerate sex chromosomes.

Additional insights into the influence of sex chromosome evolution and polyploidization may be gained from studies of laboratory-generated polyploid *Xenopus*. Laboratory allopolyploidization in *Xenopus* duplicates autosomal chromosomes but generates female polyploid individuals with 3 Z chromosomes and 1 W chromosome and male polyploid individuals with 4 Z chromosomes (Kobel and Du Pasquier 1986). Thus, the W chromosome of one of the ancestral diploids is not inherited by *Xenopus* allopolyploids, and therefore never gets a chance to segregate as an autosome. In this way, the mechanism of *Xenopus* allopolyploidization in nature may circumvent autosomal segregation of a W chromosome (whether degenerate or not) (reviewed in Evans 2008), and this could account for the unusually high incidence of polyploidization in this genus.

Analyses presented here show that changes in the system for sex chromosome turnover were much more common in amphibians than previously proposed [$\sim$32 versus 7 as proposed by Hillis and Green (1990)], and that there is not strong support for female versus male heterogamy in the ancestor of salamanders, frogs, or the most recent common ancestor of salamanders and frogs. These changes need not involve the evolution of completely novel systems for sex determination, and some of these inferred changes may be reversals to an ancestral system. We also found that changes from male to female heterogamy occurred more frequently than changes from female to male heterogamy, although the transition rates

between each state were not significantly different. This result also contradicts the conclusions of Hillis and Green (1990), who stated that there was a bias in evolution from female heterogamy to male heterogamy. One reason for these differences is that our analysis included new data on heterogamy (reviewed in Schmid et al. 2010), more species, and a more comprehensive phylogeny (Pyron and Wiens 2011). Another reason for our higher estimate in the number of changes is that we considered three de novo changes in sex determination as a new heterogamy state, thereby forcing a sex chromosome turnover in these lineages (but this is only 3 out of $\sim$32 changes). Differences in the resolution, relationships, and branch lengths of the phylogenies used in each study are also likely to have played a role in these differing conclusions. Finally, and importantly, the different analytical approaches may have influenced the results (that is, the use of maximum parsimony by Hillis and Green (1990) and maximum likelihood here).

18.4 Conclusions

Polyploidization generates new species and duplicates genes; the resulting genetic redundancy has the capacity to degrade or to undergo innovation. The question of why some lineages frequently undergo polyploidization whereas others do not thus has important implications for evolution and adaptation. Eventually we will have a much more comprehensive understanding of genetic variation in (a) the triggers of sex determination in amphibians, (b) the extent of suppressed recombination that surrounds these genetic triggers, (c) the extent of sex chromosome degeneration that exists in amphibians, and (d) whether or not other lineages of polyploid amphibians have minimally degenerate sex chromosomes. Future discoveries in these areas can undoubtedly be leveraged to provide exciting new insights into the role of sex chromosome degeneration in the propensity of species to tolerate polyploidization.

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