

Polyploidy in Amphibia

Michael Schmid^a Ben J. Evans^b James P. Bogart^c^aDepartment of Human Genetics, University of Würzburg, Würzburg, Germany; ^bDepartment of Biology, McMaster University, Hamilton, Ont., and ^cDepartment of Integrative Biology, University of Guelph, Guelph, Ont., Canada

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Allopolyploidy · Anura · Autopolyploidy · Diploidization · Hybridogenesis · Polyploidization · Sex chromosome evolution · Urodela

Abstract

This review summarizes the current status of the known extant genuine polyploid anuran and urodelan species, as well as spontaneously originated and/or experimentally produced amphibian polyploids. The mechanisms by which polyploids can originate, the meiotic pairing configurations, the diploidization processes operating in polyploid genomes, the phenomenon of hybridogenesis, and the relationship between polyploidization and sex chromosome evolution are discussed. The polyploid systems in some important amphibian taxa are described in more detail.

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Frequent Occurrence of Bisexual Polyploid Amphibians

Polyploidization has been documented across a wide range of animal taxa. These include turbellarians, annelids, mollusks, crustaceans, and insects [Gregory and Mable, 2005], teleost fishes [Schultz, 1980; Le Comber and Smith, 2004], reptiles [Gregory and Mable, 2005], and amphibians [Bogart, 1980; Kawamura, 1984; King, 1990; Schmid et al., 2010; Evans et al., 2012]. In parthe-

nogenetically reproducing animal species, polyploidy is observed relatively frequently [for reviews, see White, 1973; Fujita and Moritz, 2009]. In sexually reproducing animals, however, polyploid species are rare. Possible barriers to polyploidization include the presence of sex chromosomes [Muller, 1925], the prevalence of cross-fertilization [White, 1973], and the histological complexity of advanced animals [Stebbins, 1950]. These barriers may explain why polyploidy is much rarer in animals than in plants [Orr, 1990; Otto and Whitton, 2000].

Bisexual polyploid species of amphibians continue to be discovered. In no other class of vertebrates independently evolved polyploids are so prevalent. Unlike early chordates, including jawed vertebrates and teleost fish, where polyploidization has played a major role in ancestral evolutionary processes [Ohno, 1970, 1974; Furlong and Holland, 2002; McLysaght et al., 2002; Taylor and Raes, 2005; Mable et al., 2011] and in reptiles where polyploids are parthenogenetic [Hall, 1970; Bogart, 1980; Moritz, 1983], the Amphibia are the only vertebrates that have related diploid and polyploid bisexual species or populations. Natural amphibian polyploids have evolved independently in multiple families [Mable et al., 2011].

Autopolyploidy and Allopolyploidy

Polyploids originate by autopolyploidization (genome duplication within a species) and allopolyploidization (genome duplication associated with hybridization

among species). Individuals with autotetraploid genomes can develop by fusion of 2 diploid gametes to 1 tetraploid zygote, or by suppression of the first mitosis following fertilization. If this process is repeated, specimens with auto-octoploid karyotypes could result. In recently evolved autopolyploids, the homologous chromosomes of a quartet or octet are expected to exhibit identical chromosome banding patterns in somatic metaphases and to form multivalent pairing configurations in meiosis. In contrast, in an allopolyploid genome, if the karyotypes of the parent species are sufficiently different, then the classical banding techniques applied on the somatic metaphases should show exclusively pairs of homologous chromosomes, which all (or almost all) form bivalents in meiosis. A molecular cytogenetic approach, genomic in situ hybridization, can offer insight into a variety of interesting scenarios of allopolyploidization. A labeled genomic DNA probe of one of the diploid parental species and an unlabeled 'blocking' DNA probe from the other diploid parental species, for example, can be hybridized in situ (in an appropriate ratio) to the metaphase chromosomes of polyploid individuals. This has resulted in the detection of unexpected intergenomic exchanges in polyploid and unisexual individuals of the North American salamanders of the genus *Ambystoma* [Bi and Bogart, 2006; Bi et al., 2007a, b, 2008, 2009]. Such intergenomic exchanges generate new combinations of genes in the chromosomes, which contributes to the genetic variation and evolutionary flexibility of the unisexual individuals.

Experimental Production of Amphibian Polyploids

It is well known that polyploidy is experimentally inducible in amphibians under certain exceptional physical conditions. These conditions include temperature shock either by chilling or heat treatment of the eggs, puncturing unfertilized eggs with a fine platinum needle, and hydrostatic compression of the eggs, both in Anura [Briggs, 1947; Kawamura, 1951; Kawamura and Torkunaga, 1952; Dasgupta, 1962; Kawamura and Nishioka, 1963; Nishioka and Ueda, 1983; Kashiwagi, 1993] and Urodela [Fankhauser and Griffiths, 1939; Griffiths, 1941; Fankhauser and Watson, 1942; Fankhauser et al., 1942; Fischberg, 1944, 1945, 1948, 1958; Fankhauser and Humphrey, 1950, 1959; Jaylet, 1972; Gaillard and Jaylet, 1975; Ferrier and Jaylet, 1978]. In Urodela, these treatments produced numerous triploid and pentaploid larvae in addition to a few tetraploid and aneuploid ones. The triploid and tet-

raploid larvae sometimes completed metamorphosis, whereas the aneuploid larvae were phenotypically abnormal and died before or during metamorphosis. Fischberg [1945] was able to examine the effects of triploidy and haploidy on the sexual differentiation of the newt *Ichthyosaura alpestris*: of 27 triploid individuals, 13 with normal testes exhibited intersexual features such as overdeveloped Müllerian ducts, unpigmented Wolffian ducts, and incomplete development of secondary sexual characteristics, while 14 animals had sterile ovaries with sporadic oocytes, but were normal females with respect to the remaining sexual characteristics.

Experimentally produced amphibian triploids probably originate by inhibition of the formation or the release of the second polar body in the egg. After fertilization, the restitution nucleus is composed of 2 sets of maternal chromosomes and 1 set of paternal chromosomes. The mode of origin of the tetraploid and pentaploid individuals is unclear. In animals with a definite sex-determining mechanism, either XY♂/XX♀ or ZZ♂/ZW♀, triploidy has a striking effect on sex determination and differentiation. While the homogametic sex is normal, the heterogametic sex is changed to an intersexual condition [Bridges, 1921; Goldschmidt, 1932].

The Polyploid Amphibian Species

Previous reviews on amphibian polyploidy were published by Bogart [1980], Schmid [1980], Kawamura [1984], King [1990], Schmid et al. [2010], and Evans et al. [2012]. A wealth of data on particular species or genera and numerous references can be obtained in these reports. To the best of our knowledge, the present review contains the most complete compilation of polyploid amphibians discovered to date. Naturally occurring polyploid amphibians, including spontaneously originated and/or experimentally produced amphibian polyploids are presented in table 1. In this list, changes of family and species names that were introduced after the publication of the ploidy data are incorporated. These changes are regularly updated in the electronic database of Frost [2014]. To date, natural polyploids and spontaneously originated individuals were found to occur in 15 anuran families (Alsodidae, Arthroleptidae, Bufonidae, Ceratophryidae, Dicroglossidae, Hylidae, Leiopelmatidae, Leptodactylidae, Lymnodynastidae, Microhylidae, Odontophrynidae, Pipidae, Pyxicephalidae, Ranidae, and Strabomantidae), and in 4 urodelan families (Ambystomatidae, Plethodontidae, Salamandridae, and Sire-

Table 1. Occurrence of polyploidy in Amphibia

Order, family, species, species complex ^a	Ploidy level	References ^a
Anura		
Alsodidae		
<i>Eupsophus vertebralis</i>	3n = 42 ^j	Formas, 1993
Arthroleptidae		
<i>Astylosternus diadematus</i>	4n = 54	Bogart and Tandy, 1976, 1981
Bufonidae		
<i>Amietophrynus poweri</i>	3n = 30 ^j	Schmid, 1978
<i>Amietophrynus asmarae</i>	4n = 40	Bogart and Tandy, 1976; Bogart, 1980; Tandy et al., 1982
<i>Bufo viridis</i> complex prior to revision by Stöck et al., 2001b	3n = 33	Stöck et al., 1999, 2001a; Borkin et al., 2001; Cavallo et al., 2002
<i>Bufo viridis</i> complex prior to revision by Stöck et al., 2001b	4n = 44	Bogart, 1972; Mazik et al., 1976; Pisanets, 1978; Toktosunov, 1984; Borkin et al., 1986a, b, 2001; Orlova and Uteshev, 1986; Roth and Ráb, 1986, 1987; Wu and Zhao, 1987; Borkin and Kuzmin, 1988; Stöck, 1998; Stöck et al., 2001a, 2006
The following taxa are presently included in the <i>Bufo viridis</i> complex		
<i>Bufo baturae</i>	3n = 33	Stöck et al., 1999, 2002, 2012; Betto-Colliard et al., 2015
<i>Bufo pseudoraddei</i>	3n = 33 ^k	Stöck et al., 1999
<i>Bufo zugmayeri</i>	3n = 33 ^k	Stöck et al., 2006
<i>Bufo oblongus danatensis</i>	4n = 44	Stöck et al., 2001b, 2005
<i>Bufo oblongus oblongus</i>	4n = 44	Stöck et al., 2001b, 2005
<i>Bufo pewzowi pewzowi</i>	4n = 44	Stöck et al., 2001b, 2005, 2010; Betto-Colliard et al., 2015
<i>Bufo pewzowi strauchii</i>	4n = 44	Stöck et al., 2001b, 2005, 2010
<i>Bufo pewzowi taxkorensis</i>	4n = 44	Stöck et al., 2001b, 2005, 2010
<i>Bufo pewzowi unicolor</i>	4n = 44	Stöck et al., 2001b, 2005, 2010
Ceratophryidae		
<i>Ceratophrys aurita</i>	8n = 104	Beçak, 1967; Beçak et al., 1967
<i>Ceratophrys ornata</i>	8n = 104	Bogart, 1967; Barrio and Rinaldi de Chieri, 1970a; Bogart and Wasserman, 1972
<i>Ceratophrys joazeirensis</i>	8n = 104	Vieira et al., 2006
Dicroglossidae		
<i>Hoplobatrachus occipitalis</i>	4n = 52	Bogart and Tandy, 1976, 1981
Hylidae		
<i>Hyla versicolor</i>	4n = 48	Wasserman, 1970; Bogart and Wasserman, 1972; Bachmann and Bogart, 1975; Cash and Bogart, 1978; Wiley, 1982; Anderson, 1986, 1991
<i>Hyla versicolor</i> × <i>H. chrysoscelis</i> ^b	3n = 36	Bogart and Bi, 2013
<i>Phyllomedusa tetraploidea</i>	4n = 52	Beçak et al., 1970b; Batistic et al., 1975; Pombal and Haddad, 1992; Haddad et al., 1994
Leiopelmatidae		
<i>Leiopelma hochstetteri</i>	3n = 33 ^j	Green et al., 1984
Leptodactylidae		
<i>Pleurodema bibroni</i>	4n = 44	Barrio and Rinaldi de Chieri, 1970b; Kuramoto, 1972; Veloso et al., 1973
<i>Pleurodema kriegi</i>	4n = 44	Barrio and Rinaldi de Chieri, 1970b; Bogart and Wasserman, 1972
<i>Pleurodema cordobae</i>	8n = 88	Valetti et al., 2009
Lymnodynastidae		
<i>Neobatrachus aquilonius</i>	4n = 48	Mahony and Roberts, 1986
<i>Neobatrachus centralis</i>	4n = 48	Mahony and Roberts, 1986
<i>Neobatrachus kunapalari</i>	4n = 48	Mahony and Roberts, 1986
<i>Neobatrachus sudelli</i>	4n = 48	Mahony and Robinson, 1980; Mahony and Roberts, 1986
Microhylidae		
<i>Aphantophryne pansa</i>	4n = 52	Kuramoto and Allison, 1989
<i>Chiasmocleis leucosticta</i>	4n = 48	Kasahara and Haddad, 1997
<i>Scaphiophryne gottlebei</i>	4n = 52	Vences et al., 2002

Table 1 (continued)

Order, family, species, species complex ^a	Ploidy level	References ⁿ
Odontophrynidae <i>Odontophrynus americanus</i>	4n = 44	Beçak et al., 1966, 1967, 1970a; Bogart, 1967; Martino and Sinsch, 2002
Pipidae		
Species with <i>Xenopus tropicalis</i> -type karyotypes (<i>Silurana</i> group)		
<i>X. tropicalis</i>	3n = 30 ^j	Schmid and Steinlein; this issue
<i>X. epitropicalis</i>	4n = 40	Tymowska and Fischberg, 1982; Tymowska, 1991
<i>X. new tetraploid 1</i>	4n = 40	Tymowska, 1991; Evans et al., 2004
<i>X. new tetraploid 2</i>	4n = 40	Evans et al., 2004
Species with <i>Xenopus laevis</i> -type karyotypes (<i>Xenopus</i> group)		
<i>X. borealis</i>	4n = 36	Tymowska and Fischberg, 1973; Tymowska, 1976, 1977, 1991
<i>X. clivii</i>	4n = 36	Tymowska and Fischberg, 1973; Tymowska, 1977
<i>X. fraseri</i>	4n = 36	Tymowska and Fischberg, 1973; Tymowska, 1977
<i>X. gilli</i>	4n = 36	Tymowska and Fischberg, 1973; Tymowska, 1977, 1991
<i>X. laevis</i>	4n = 36	Wickbom, 1945; Weiler and Ohno, 1962; Morescalchi, 1963; Tymowska and Kobel, 1972; Tymowska, 1977, 1991
<i>X. largeni</i>	4n = 36	Tymowska, 1991
<i>X. muelleri</i>	4n = 36	Tymowska and Kobel, 1972; Tymowska and Fischberg, 1973; Tymowska, 1991
<i>X. petersi</i> ^{c,d}	4n = 36	Tymowska and Fischberg, 1973; Tymowska, 1977, 1991
<i>X. poweri</i> ^d	4n = 36	Tymowska, 1991
<i>X. pygmaeus</i>	4n = 36	Loumont, 1986
<i>X. sp. nov. VI</i> ^e	4n = 36	Tymowska, 1991
<i>X. victorianus</i> ^d	4n = 36	Tymowska and Fischberg, 1973; Tymowska, 1977, 1991
<i>X. amieti</i>	8n = 72	Kobel et al., 1980
<i>X. andrei</i>	8n = 72	Loumont, 1983
<i>X. boumbaensis</i>	8n = 72	Loumont, 1983
<i>X. itombwensis</i> ^f	8n = 72	Evans et al., 2008
<i>X. lenduensis</i>	8n = 72	Evans et al., 2011
<i>X. vestitus</i>	8n = 72	Tymowska, 1976; Tymowska et al., 1977
<i>X. wittei</i>	8n = 72	Tymowska, 1976; Tymowska and Fischberg, 1980
<i>X. sp. nov. X</i>	8n = 72	Tymowska, 1991
<i>X. longipes</i>	12n = 108	Loumont and Kobel, 1991
<i>X. ruwenzoriensis</i>	12n = 108	Tymowska and Fischberg, 1973; Tymowska, 1991
<i>X. cf. boumbaensis</i>	12n = 108	Evans, 2007
<i>X. sp. nov. VIII</i> ^g	12n = 108	Tymowska, 1991
Pyxicephalidae		
<i>Tomopterna tandyi</i>	4n = 52	Bogart and Tandy, 1976, 1981; Channing and Bogart, 1996
Ranidae		
<i>Glandirana rugosa</i>	3n = 39 ^l	Kashiwagi, 1993
<i>Lithobates pipiens</i>	3n = 39 ^l	Briggs, 1947
<i>Lithobates chiricahuensis</i> × <i>L. pipiens</i> ^b	3n = 39	Green and Delisle, 1985
<i>Pelophylax esculentus</i> ^h	3n = 39	Uzzell and Berger, 1975; Heppich, 1978; Heppich and Tunner, 1979
<i>Pelophylax esculentus</i> ^h	4n = 52 ^j	Borkin et al., 2004
<i>Pelophylax esculentus</i> ^h	5n = 65 ^j	Hermaniuk et al., 2013
<i>Pelohylax nigromaculatus</i>	3n = 39 ^l	Kawamura, 1951
<i>Pelohylax nigromaculatus</i>	4n = 52 ^l	Kawamura, 1939
<i>Pelohylax nigromaculatus</i>	6n = 78 ^l	Kawamura, 1939
<i>Rana japonica</i>	3n = 39 ^l	Kawamura and Tokunaga, 1952
Strabomantidae		
<i>Holoaden luederwaldti</i>	3n = 27 ^j	Campos et al., 2012

Table 1 (continued)

Order, family, species, species complex ^a	Ploidy level	References ⁿ
Urodela		
Ambystomatidaeⁱ		
<i>Ambystoma mexicanum</i>	3n = 42 ^l	Fankhauser and Humphrey, 1950, 1959
<i>Ambystoma mexicanum</i>	4n = 56 ^l	Fankhauser and Humphrey, 1959
<i>Ambystoma nothagenes</i> (unisexual LTTi)	3n = 42	Kraus, 1985
<i>Ambystoma platineum</i> (unisexual LJJ)	3n = 42	Uzzell, 1963, 1964; Sessions, 1982
<i>Ambystoma tremblayi</i> (unisexual LLJ)	3n = 42	Uzzell, 1963, 1964; Sessions, 1982
<i>Ambystoma</i> (unisexual LLT)	3n = 42	Bogart et al., 1985
<i>Ambystoma</i> (unisexual LTT)	3n = 42	Downs, 1978
<i>Ambystoma</i> (unisexual LLLT)	4n = 56	Bogart and Licht, 1986
<i>Ambystoma</i> (unisexual LLTT)	4n = 56	Bogart and Licht, 1986
<i>Ambystoma</i> (unisexual LTTT)	4n = 56	Bogart and Licht, 1986
<i>Ambystoma</i> (unisexual LLLJ)	4n = 56	Bogart and Klemens, 1997
<i>Ambystoma</i> (unisexual LLJJ)	4n = 56	Bogart and Klemens, 1997
<i>Ambystoma</i> (unisexual LJJJ)	4n = 56	Morris and Brandon, 1984
<i>Ambystoma</i> (unisexual LJJB)	4n = 56	Bogart et al., 2009
<i>Ambystoma</i> (unisexual LTTi)	4n = 56	Bogart, 2003
<i>Ambystoma</i> (unisexual LTTTi)	4n = 56	Bogart et al., 1987
<i>Ambystoma</i> (unisexual LLLLJ)	5n = 70	Lowcock and Murphy, 1991
Plethodontidae		
<i>Eurycea bislineata</i>	3n = 42 ^j	Fankhauser, 1939
<i>Eurycea bislineata</i>	4n = 56 ^j	Fankhauser, 1939
Salamandridae		
<i>Cynops pyrrhogaster</i>	3n = 36 ^{i,1}	Fankhauser et al., 1942
<i>Ichthyosaura alpestris</i>	3n = 36 ^l	Fischberg, 1944, 1945, 1948, 1958
<i>Lissotriton vulgaris</i>	3n = 36 ^j	Böök, 1940; Litvinchuk et al., 1998
<i>Notophthalmus viridescens</i>	3n = 36 ^{i,1}	Fankhauser and Griffiths; 1939; Fankhauser, 1941; Griffiths, 1941; Fankhauser and Watson, 1942
<i>Pleurodeles waltl</i>	3n = 36 ^l	Jaylet, 1972; Gaillard and Jaylet, 1975; Ferrier and Jaylet, 1978
Sirenidae		
<i>Pseudobranchius striatus</i>	4n = 48 ^m	Morescalchi et al., 1986
<i>Pseudobranchius striatus</i>	6n = 64 ^m	Morescalchi and Olmo, 1974; Morescalchi et al., 1986
<i>Siren intermedia</i>	4n = 46	Morescalchi and Olmo, 1974; Morescalchi et al., 1986
<i>Siren lacertina</i>	4n = 52	Morescalchi and Olmo, 1974; Morescalchi, 1975
<p>Included are the naturally occurring polyploid species and spontaneously originated and/or experimentally produced polyploid individuals.</p> <p>^a Changes of family and species names introduced after publication of the ploidy data were taken into consideration and are regularly updated in the electronic database of Frost [2014].</p> <p>^b Natural allotriploid hybrids.</p> <p>^c Species called <i>X. sp. nov. IX</i> by Tymowska [1991].</p> <p>^d For nomenclature, see Furman et al. [2015].</p> <p>^e Species called 'X. new tetraploid' by Evans et al. [2004].</p> <p>^f In the review of Schmid et al. [2010], this species is erroneously listed as dodecaploid.</p> <p>^g According to Evans et al. [2012], this dodecaploid species status may be the same as <i>X. cf. boumbaensis</i>; however, unpublished data [B.J. Evans] indicates that these 2 dodecaploid species are in fact each a distinct species.</p> <p>^h <i>Pelohylax esculentus</i> is a hybrid of <i>Pelohylax lessonae</i> and <i>Pelohylax ridibundus</i>.</p> <p>ⁱ Unisexual, all-female salamanders in the North American genus <i>Ambystoma</i> are mostly polyploid and contain nuclear genomes of 2–4 different species that can include <i>Ambystoma laterale</i> (L), <i>A. jeffersonianum</i> (J), <i>A. texanum</i> (T), <i>A. tigrinum</i> (Ti), or <i>A. barbouri</i> (B). The unisexuals all have a similar mtDNA and always include at least 1 L genome in their nuclei. The various genomic combinations and the reproductive system used by unisexual <i>Ambystoma</i> are described by Bogart [2003] and Bogart et al. [2007, 2009].</p> <p>^j Spontaneously originated polyploid individual(s) found in wild populations.</p> <p>^k Unclear whether the entire taxon is polyploid or only many individuals in the population.</p> <p>^l Experimentally obtained polyploid individuals.</p> <p>^m Results not supported by analyses of Moler and Kezer [1993].</p> <p>ⁿ Only the relevant initial cytogenetic studies, revisions and reviews are listed.</p>		

nidae). It seems that polyploidy is a widespread phenomenon in the orders Anura and Urodela, that has played a role in speciation and evolution. No polyploids have yet been found in species of the order Gymnophiona, but compared to anurans and urodelans, relatively low numbers of individuals have been cytogenetically analyzed.

***Bufotes viridis* Complex**

Palaearctic toads of the *Bufotes viridis* complex (table 1) form a monophyletic radiation of at least 12 major mitochondrial DNA haplotype groups [Stöck et al., 2006]. There are several cases of range overlap and interactions through hybridization and polyploidization [Colliard et al., 2010; Dufresnes et al., 2014]. This radiation includes bisexually reproducing diploid ($2n = 22$), triploid ($3n = 33$) and tetraploid ($4n = 44$) species, all of which occur in Central Asia [Stöck et al., 2010]. Of particular interest was the discovery of the all-triploid anuran species *Bufotes baturae* (table 1) [Stöck et al., 1999, 2002, 2012; Betto-Colliard et al., 2015]. This toad species maintains a pure triploid status by an exceptional mechanism that modifies meiosis in males and females in such a way that both parents contribute unequal amounts of their genomes ($n_{\sigma} + 2n_{\phi}$) to their triploid offspring [Stöck et al., 2002, 2012; Betto-Colliard et al., 2015]. In other vertebrates, the very rare occurrence of triploidy is coupled with infertility or unisexuality, or requires the coexistence of individuals with different degrees of ploidy in the reproductive community.

Genera *Ceratophrys*, *Odontophrynus* and *Pleurodema*

South American frogs belonging to the families Ceratophryidae, Odontophrynidae and Leptodactylidae were among the first naturally occurring polyploid species found in vertebrates (table 1). Saez and Brum [1960] observed high chromosome numbers in *Odontophrynus americanus* and *Ceratophrys ornata* but did not realize their polyploid nature. Few years later, Beçak et al. [1966] recognized the tetraploid status of *O. americanus*. Extensive studies on many populations of *O. americanus* in Brazil, Uruguay and Argentina showed that this species consists of diploid ($2n = 22$) and tetraploid ($4n = 44$) populations [Beçak et al., 1967, 1970a; Bogart, 1967; Barrio and Rinaldi de Chieri, 1970a; Barrio and Pistol de Rubel, 1972]. The tetraploid *O. americanus* is now considered to

be included in a complex of species that contain diploid members [Rosset et al., 2006; Grenat et al., 2009]. Beçak et al. [1967] demonstrated that the Brazilian *Ceratophrys dorsata* (now *C. aurita*) is an octoploid species ($8n = 104$) probably derived from diploid ceratophryids with $2n = 26$ chromosomes. In *C. ornata*, another species of this genus from Argentina, both octoploid as well as diploid populations were discovered [Bogart, 1967; Bianchi and Molina, 1968; Barrio and Rinaldi de Chieri, 1970a; Bogart and Wasserman, 1972]. Later, the diploid *C. ornata* was described as a distinct species, *C. cranwelli*, by Barrio [1980], and a third octoploid species, *C. joazeirensis*, was reported by Mercadal de Barrio [1986]. Clearly, many of the previously recognized diploid-polyploid populations are, in reality, diploid and polyploid cryptic species, which detract from the notion that polyploidy may arise spontaneously as an artifact in populations of diploid individuals. To better understand the evolution of ploidy levels in this group, it will be useful to know if the 3 species of *Ceratophrys* had an octoploid common ancestor or if they evolved independently from diploids, tetraploids, or perhaps hexaploids.

Comparative meiotic analyses in male and female individuals of diploid ($2n = 22$) and tetraploid ($2n = 44$) *O. americanus*, diploid *C. cranwelli* ($2n = 26$) and octoploid *C. ornata* ($2n = 104$) showed that diploid females form more chiasmata in the paired chromosome arms than diploid males and polyploids of both sexes [Rahn and Martínez, 1983].

It has been assumed that the polyploid populations of the South American *Odontophrynus* and *Ceratophrys* evolved recently by autopolyploidization [Beçak et al., 1967, 1970a, b; Bogart, 1980]. Their conventionally stained karyotypes show homogeneity within the chromosome groups, and the homologous chromosomes still pair as multivalents in meiosis. In accordance with this, electrophoretic analyses on 9 enzyme systems yielded no detectable polymorphisms in tetraploid *O. americanus* [Schwantes, 1974]. However, the results obtained by C-banding show that distinct heterogeneities exist in the banding patterns in the quartets 1–4, 6 and 11 of *O. americanus* and the octets 2, 4 and 5 of *C. ornata* [Schmid et al., 1985]. In the chromosomes of the quartets 3 and 4 of *O. americanus*, there are even slight differences in the arm ratios, which are visible in conventionally stained preparations. Ruiz et al. [1981] performed an extensive cytogenetic study on several diploid and tetraploid populations in the *O. americanus* complex from Brazil and Uruguay. These authors also found a tetraploid individual in which 2 of the chromosomes of the quartet 4 can

be differentiated from the 2 other homologues by the position of constitutively heterochromatic regions. It is remarkable that C-banding subdivides each of the quartets 3, 4 and 11 of *O. americanus* into exactly 2 pairs of homologous chromosomes and each of the octets 2, 4 and 5 of *C. ornata* into 2 quartets of homologues. The conclusion could be drawn that these frogs are not autopolyploids as was previously assumed, but rather are of hybrid (allopolyploid) origin. But, the high frequency of meiotic multivalent pairing configurations in *O. americanus* and *C. ornata*, which are characteristic for autopolyploid organisms, brings this inference into question. The alternative explanation is that the differences in the banding patterns within the quartets and octets in question developed gradually after autopolyploidization. It is known that polyploid genomes generally become functionally diploid in the course of evolution (diploidization, see below).

Genus *Xenopus*

Polyploid species of the African clawed frogs of the genus *Xenopus* (table 1) are thought to have an entirely (or almost entirely) hybrid (allopolyploid) origin [for review, see Evans, 2008]. Amazingly, polyploid species appear to have originated independently by allopolyploidization on multiple occasions, with tetraploids arising twice (once in the *Silurana* group and once in the *Xenopus* group as defined by Kobel et al. [1996]), octoploids arising at least 3 times, and dodecaploids arising at least 3 times [Evans, 2007]. Unpublished data (B.J. Evans) has identified additional dodecaploid species and suggests that the actual number of independent polyploidization events is even higher than this. In these species, the analyses of male meiosis demonstrate almost exclusively bivalents [Tymowska, 1991]. The rare multivalent pairing configurations probably have modest genomic repercussions in terms of crossover events between the duplicated pairs of homologous chromosomes per generation, and are not more frequent than in experimentally produced hybrids [Müller, 1977]. Analyses of the conventionally stained tetraploid karyotype of *X. epitropicalis* have shown that the chromosomes can be grouped into sets of 4 chromosomes (quartets). But, C-banding revealed heterogeneity of the constitutive heterochromatin within all quartets, dividing each of them into 2 pairs of homologous chromosomes [Tymowska and Fischberg, 1982]. In contrast to C-banding, high-resolution BrdU/dT-replication bands in metaphase chromo-

somes of diploid *X. tropicalis* ($2n = 20$) and both tetraploid *X. epitropicalis* and *X. new* tetraploid 1 ($4n = 40$) showed the existence of perfect replication homoeologies in all 10 chromosome pairs and quartets of the 3 species; no apparent replication asynchronies could be detected [Schmid and Steinlein, this issue]. Considering the allopolyploid origin of *X. epitropicalis* and *X. new* tetraploid 1, this observation supports the conclusion that the 2 ancestral diploid parental species were closely related with extremely similar, if not identical, karyotypes. The replication banding patterns in their euchromatic chromosome regions were still the same, whereas the fast-evolving and genetically inert constitutive heterochromatin had already diverged.

Hyla versicolor – *H. chrysoscelis* Complex

Tetraploid *Hyla versicolor* (table 1) and diploid *H. chrysoscelis* are cryptic species that are widely distributed in eastern North America [Bogart, 1980]. The 2 species cannot be distinguished by morphology, but they have distinctly different vocalizations that were originally used to differentiate them [Johnson, 1966] prior to their identification as a diploid (*H. chrysoscelis*) and tetraploid (*H. versicolor*) species pair [Wasserman, 1970; Bogart and Wasserman, 1972]. Based on mitochondrial sequences [Ptacek et al., 1994], *H. versicolor* arose at least 3 times, twice from *H. chrysoscelis* and once from an unknown maternal ancestor. Using mitochondrial and nuclear markers, Holloway et al. [2006] also suggested that tetraploids arose multiple times from *H. chrysoscelis* and from 2 other extinct lineages. Rather than tetraploidy arising de novo from diploid ancestors, the available mitochondrial data would also support a ‘triploid bridge hypothesis’ [Ralin and Selander, 1979; Bogart and Bi, 2013] where new tetraploids arise from triploid hybrids.

Terraranean Frogs

The unranked anuran taxon Terrarana is an immense group of frogs that includes the 5 families Brachycephalidae, Ceuthomantidae, Craugastoridae, Eleutherodactylidae, and Strabomantidae [Hedges et al., 2008; Heinicke et al., 2009] and nearly 1,000 recognized species, which is one sixth of the 5,984 known species of anurans [AmphibiaWeb, 2015]. New species of terraranans are being discovered and described at an extraordinary rate of about 15–25 species per year. In a monograph on terra-

ranan frogs, 2,548 specimens belonging to 227 species of Terrarana were cytogenetically examined [Schmid et al., 2010]. Furthermore, all the previously published cytogenetic analyses on at least 1,673 terraranan specimens of 138 species were reevaluated (many of the early reports do not indicate the number of specimens examined). It came as a surprise that all data accumulated in 2010 indicated that polyploidy seems to be absent in the huge taxon Terrarana. Not a single polyploid individual was found among a total of at least 4,221 cytogenetically examined terraranan specimens. The only terraranan species in which the existence of polyploidy had ever been discussed is the complex case of *Haddadus binotatus* from Brazil [Beçak and Beçak, 1974]. This craugastorid has a moderate diploid chromosome number of $2n = 22$, but its chromosomes are by far the largest (in terms of length, width and degree of condensation) of all terraranan species so far examined. Concomitantly, the genome size of this species (27 pg DNA/nucleus) is the largest in the taxon, the nuclear volume in erythrocytes is distinctly larger than in the other terraranans, and multivalent ring configurations (up to dodecavalents) are frequently formed in male meiosis. These observations led Beçak and Beçak [1974] to consider the possibility that the karyotype of *H. binotatus* originated by polyploidization combined with intercalary duplications. Subsequently, the chromosome number was reduced by non-reciprocal translocations as indicated by the multiple meiotic multivalent ring configurations. But all the examined species of the family Craugastoridae possess diploid chromosome numbers in the range of $2n = 18$ to $2n = 24$ and fundamental numbers in the range of $FN = 32$ to $FN = 44$, which are very similar values to those of *H. binotatus* ($2n = 22$, $FN = 38$). Therefore, it is unlikely that the *H. binotatus* karyotype was derived from a polyploid ancestor that experienced a series of multiple non-reciprocal translocations. It is more conceivable that the unusually large genome of *H. binotatus* is the result of considerable intercalary amplification of middle repetitive DNA sequences, as has already been demonstrated to exist in other Amphibia. An experimental approach to test this possibility is to subject *H. binotatus* DNA to re-association kinetic experiments [Baldari and Amaldi, 1976]. Nevertheless, as has been shown by Siqueira et al. [2004] and Schmid et al. [2010], a translocation heterozygosity involving 2 chromosome pairs exists in the *H. binotatus* population. This reciprocal translocation, however, only accounts for the meiotic ring quadrivalents and not for multivalents with more than 4 involved chromosomes. Therefore, it has been suggested [Siqueira et al.,

2004] that multiple reciprocal and terminal translocations between non-homologous chromosomes are present in the *H. binotatus* karyotype. Such small reciprocal translocations between terminal chromosome regions have also been proposed for explaining the multivalent configurations in male meiosis of the frog *Physalaemus petersi* [Lourenço et al., 2000]. More recently, meiotic ring multivalents, with the participation of nucleolus organizer region (NOR)-bearing chromosomes, have been described for the Brazilian hyliid frogs *Aplastodiscus albofrenatus* and *A. arildae*, which were again traced back to multiple terminal translocations [Carvalho et al., 2009]. An alternative and simple explanation for such terminal meiotic associations between non-homologous bivalents is non-chiasmatic ectopic pairing between heterochromatic telomeric regions. Such terminal associations are common in many animal species as has been shown by Drets and Stoll [1974] and John and King [1982, 1985]. Furthermore, Callan [1991] has demonstrated that the telomeres of homologous, but also of non-homologous chromosomes, are frequently fused to one another, but dissociate by first meiotic metaphase in meiosis of diploid urodelan and anuran species. Despite the presumption that polyploidy may be absent in terraranans, Campos et al. [2012] reported the first spontaneous triploid individual in the strabomantid *Holoaden luederwaldti* (table 1). Among 7 individuals (5♂, 2♀) collected in the Atlantic Forests of Brazil (Campos de Jordão municipality), 6 were diploid ($2n = 18$) and 1 male was triploid ($3n = 27$) in all somatic tissues analyzed. The preparations obtained from testes showed few unidentifiable meiotic stages. No morphological difference between the diploid specimens and the triploid animal was apparent. Taking together the sampling numbers in Schmid et al. [2010] and the finding of Campos et al. [2012], the proportion of spontaneous polyploids in natural terraranan populations under normal environmental conditions is approximately 1:4,200 (~0.025%). This proportion has limited extension to other amphibians that inhabit temperate zones of the northern hemisphere and develop during spring when temperatures can drop abruptly to temperatures below 0°C. As described above, chilling of amphibian eggs can induce polyploidy. Therefore, the rate of spontaneously developing polyploids in these species can be distinctly higher than in the neotropical Terrarana. Thus, Richards and Nace [1977] detected varying and occasionally large numbers of unreduced diploid eggs laid by individual female specimens of the frog *Lithobates pipiens* and concluded that these eggs, when fertilized, would give rise to triploid embryos.

***Pelohylax esculentus* – *P. lessonae* – *P. ridibundus* Complex**

Hybridization of 2 diploid species may lead to polyploidy, because differences between 2 divergent genomes can lead to meiotic disturbances and, in turn, to aneuploid gametes [Vrijenhoek, 1989]. Thus, single cases of viable allotriploid hybrids were detected among offspring of natural crosses between the frogs *Lithobates chiricahuensis* and *L. pipiens* and between *H. versicolor* and *H. chrysoscelis* (table 1). An unusual case of polyploidy occurs in the frog *Pelophylax esculentus* (table 1). This amphibian exists in numerous diploid and triploid populations from France eastward into European Russia and from southern Scandinavia into Italy. *P. esculentus* is a hybrid between the bisexual species *P. lessonae* (LL genome) and *P. ridibundus* (RR genome) and is sympatric with the parental species over much of its range. Both species and the hybrid can be freely crossed in laboratory conditions, giving progeny of varying viability [Berger, 1988; Berger et al., 1994]. Diploid individuals (LR genome) depend on gametes from one or the other parental species. In the *lessonae-esculentus* system, frogs exclude the L genome during gametogenesis and produce exclusively clonal R gametes (hybridogenesis, hemiclinal reproduction). Therefore, they have to cross with *P. lessonae* to produce new hybrids [for reviews, see Graf and Polls Pelaz, 1989; Christiansen, 2009]. Interhybrid crossings yield RR offspring that usually die before reaching sexual maturity, because of homozygosity for deleterious mutations [Vorburger, 2001; Guex et al., 2002; Christiansen, 2009]. A reverse form of this system operates in the *ridibundus-esculentus* system [for reviews, see Graf and Polls Pelaz, 1989; Christiansen, 2009]. Here LR frogs predominantly produce L gametes and, therefore, have to cross with *P. ridibundus* in order to form new hybrids.

P. ridibundus does not always exist in stable diploid systems. In many geographic regions, the diploid parental species (LL and RR genomes), diploid hybrids (LR genomes), and triploid hybrids (LLR and LRR genomes) are present [Rybacki and Berger, 2001]. In the northern part of the range (Sweden, Denmark, northern Germany, northern Poland), diploids and triploids often form all-hybrid populations [Regnier and Neveu, 1986; Zavadil, 1994; Lada et al., 1995; Mikulíček and Kotlík, 2001; Rybacki and Berger, 2001; Christiansen et al., 2005; Arioli, 2007; Jakob, 2007; Christiansen and Reyer, 2009; Arioli et al., 2010]. Spontaneous cases of tetraploidy were reported in some populations, but it is not known if they are of any importance for the genetic dynamics of these populations

[Borkin et al., 2004; Christiansen, 2009]. A detailed overview on the hybridogenic reproduction mode of *P. esculentus* in the various populations, the types, ploidy degree and frequencies of different gametes, as well as the XY sex-determining system was published by Christiansen et al. [2005] and Christiansen [2009].

Genus *Neobatrachus*

There are 9 extant species of Australian lymnodynastid frogs in the genus *Neobatrachus*. 5 species (*N. fulvus*, *N. pelobatooides*, *N. pictus*, *N. sutor*, *N. wilsmorei*) are diploid ($2n = 24$), and 4 species (*N. aquilonius*, *N. centralis*, *N. kunapalari*, *N. sudelli*) are tetraploid ($4n = 48$; table 1). The 4 tetraploid species inhabit wide areas of Australia allopatrically. According to Mahony and Roberts [1986], the origin of the tetraploids was by autopolyploidy in a single event. They did not find any indication of diploidization (see below) in the tetraploid karyotypes. In the diakinesis stage of meiosis, in male tetraploids a high frequency of tetravalents was observed; 70% of the diakinesis contained more tetravalents than bivalents. Some of the diploid species occur sympatrically with the tetraploid species, and in 2 localities, triploid hybrids were detected. Additionally, a single pentaploid specimen was found.

Diploidization of Polyploid Genomes

Diploidization processes operating in polyploid genomes cause divergent development of originally identical chromosomes and genes. They lead to strict formation of bivalents in meiosis and disomic segregation rates. Diploidized genomes are polyploid with respect to the amount of genetic material and the number of gene copies, but diploid with respect to the nature of cell division in that each chromosome pairs with only 1 other partner [Ohno, 1970; Ferris and Whitt, 1977; Leipoldt, 1983; Soltis and Soltis, 1999; Wendel, 2000; Gregory and Mable, 2005; Braasch and Postlethwait, 2012; Roulin et al., 2012]. Diploidization must have been important to stabilize the benefits associated with polyploidization, such as the creation of genes with the potential to evolve novel functions [Ohno, 1970]. For the tetraploid Asian bufonid species and subspecies (table 1) both allopolyploid and autopolyploid modes of origin have been proposed, but this is still controversial. Whether the presence of unusual quinacrine-bright heterochromatic bands in single chromosomes of these tetraploid bufonid species and subspecies

reflects an ancestral polymorphism of constitutive heterochromatin, or is evidence of diploidization, or is a sign of hybridization between different taxa (as closely related mitochondrial DNA sequences in diploid and tetraploid specimens suggest), is still unknown [for review, see Stöck et al., 2005].

The preferential formation of bivalents instead of multivalents is the prerequisite for diploidization. Therefore, a structural heterogeneity must be created among the homologous chromosomes in the polyploid karyotype. Careful studies on the karyotypes of phylogenetically tetraploid fishes of the orders Isospondyli and Ostariophysi have shown that such structural heterogeneities can originate by centric fusions and pericentric inversions of the chromosomes [Ohno, 1970, 1974; Ohno et al., 1968]. It has been concluded that much smaller changes than the inter- and intrachromosomal rearrangements mentioned above suffice to initiate the diploidization process [Schmid et al., 1985]. These definite differences between the original homologues in some quartets of *O. americanus* and octets of *C. ornata* are manifested in the size, staining intensity, and position of some C-bands. It is generally accepted that constitutively heterochromatic regions are enriched with genetically inert DNA [Arrighi and Saunders, 1973]. Furthermore, closely related species may possess entirely different satellite DNAs [Walker, 1968; Hennig and Walker, 1970; Arrighi and Saunders, 1973], as well as different C-banding patterns in their karyotypes. Apparently, the evolutionary changes occurring in the highly repeated DNA sequences located in the constitutive heterochromatin can be very rapid because they are not subject to selective pressure. This could also explain why some quartets and octets of *O. americanus* and *C. ornata*, which are supposed to be recently evolved autopolyploids, show heterogeneities in their heterochromatin patterns even though other chromosome parameters (length, arm ratio, constrictions) are usually unchanged.

The number of active NORs is important with respect to a possible diploidization of the gene activity in the genomes of polyploids. In *O. americanus*, the 4 NORs in the quartet 11 correspond to the tetraploid status [Schmid et al., 1985]. In agreement with this, by means of molecular RNA-DNA-hybridization experiments, Schmidtke et al. [1976] have found that tetraploid *O. americanus* are endowed with about twice the number of 18S + 28S ribosomal DNA (rDNA) cistrons than the diploid frogs. In contrast to this, comparative measurements of erythrocyte volume and hemoglobin content per cell, lactate dehydrogenase activity in heart muscle tissue [Beçak and

Pueyo, 1970], and RNA content per kidney cell [Beçak and Goissis, 1971] essentially yield identical values in the tetraploid and diploid *O. americanus*. This would suggest that overall levels of gene expression in tetraploids may be similar to those of diploids. Various observations in animals [Pedersen, 1971] and plants [Maher and Fox, 1973; Siegel et al., 1973] indicate a selective loss of 18S + 28S rDNA cistrons as a possible means of decreasing genetic activity. In *O. americanus*, however, the phenomenon of diploidization of genetic activity cannot be attributed to the loss of 18S + 28S rDNA as shown by the number of active NORs and the amount of rDNA present in the genomes. This result corresponds with the findings in diploid and tetraploid species of the fish family Cyprinidae. While genetic activity is similarly diploidized in the tetraploids [Schmidtke and Engel, 1975], the number of 18S + 28S rDNA cistrons is proportional to the degree of ploidy [Schmidtke et al., 1975]. A similar mechanism might likewise be responsible for the reduction of the genetic activity observed in tetraploid *O. americanus* [Beçak and Pueyo, 1970; Beçak and Goissis, 1971]. A study on the methylation status of the 18S + 28S rDNA cistrons in *O. americanus* revealed that methylation of the ribosomal genes was increased in the tetraploid genomes of adult animals, but exact quantitative determinations could not be obtained [Ruiz and Brisson, 1989]. In contrast to the results in *O. americanus*, the cytogenetically examined specimens of *C. ornata* did not exhibit a NOR number that corresponded to its octoploid status [Schmid et al., 1985]. The NOR-specific Ag-staining, mithramycin- and chromomycin A₃-fluorescence unequivocally showed that 4 NORs had been deleted in octet 7. Similarly, most of the frogs possessed only 4 NORs in octet 8, and more than 4 NORs were rare. This loss of rRNA genes can be interpreted as a sign of progressive diploidization. As concluded by Schmid et al. [1985], further studies on other octoploid populations of *C. ornata* are necessary to find out if there are still individuals showing the maximum number of 16 NORs in their karyotypes, as well as the various intermediary stages of NOR reduction. The diploidization of the rRNA genes in *C. ornata* would be concluded if only 1 pair of NORs were present in octets 7 and 8. In all tetraploid Asian bufonid species and subspecies (table 1), only 2 NORs have been detected by Ag-staining, mithramycin staining, as well as by 18S + 28S rDNA in situ hybridization [Stöck et al., 2005]. This demonstrates an actual loss of 50% of the original ribosomal cistrons of the tetraploid frog genomes and is an indication of an advanced diploidization process. Also tetraploid *Xenopus* species have

a single pair of nucleolar constrictions and a single pair of Ag-stained NORs in their karyotypes; co-dominant expression of the parental NORs, which is expected in polyploids, has not been found in tetraploid *Xenopus* species [Tymowska and Fischberg, 1982; Tymowska, 1991]. This indicates either a genetic inactivation or, more probably, a complete loss of the other ribosomal cistrons.

Finally, it must be emphasized that, in addition to the process of diploidization, considerable chromosomal rearrangements, as deletions, and inversions, intra- and intergenomic translocations and insertions can occur after polyploidization and change the karyotype in relatively short time [Soltis and Soltis, 1999; Adams, 2007; Gaeta et al., 2007].

Sex Chromosome Evolution and Polyploidization

In amphibians, sex determination is triggered by allelic differences between sex-specific and non-sex-specific regions of the sex chromosomes. Sex specificity is thought to be maintained by a lack of genetic recombination between a portion of the chromosome that carries the genetic trigger (the Y or W) and the other sex chromosome that is not sex-specific (the X or Z, respectively) [Charlesworth et al., 2005; Bergero and Charlesworth, 2009]. Interestingly, in some species the 'sex-specific' region in fact is not always sex-specific, and this could limit the extent of sex chromosome divergence if there exist differences in the rate of recombination between phenotypic males and females [Perrin, 2009; Stöck et al., 2011].

If the non-recombining region encompasses a large portion of the sex chromosome, each allele of other genes (that do not trigger sex determination) also has an independent evolutionary fate on each sex chromosome. Occasionally, an allele of one of these genes in the non-recombining portion of the sex-specific chromosome becomes a pseudogene, creating an imbalance between the sexes in the number of alleles. Loss of alleles on the sex-specific chromosome is potentially problematic for a polyploid genome if 1 copy of this chromosome acquires an autosomal mode of inheritance after genome duplication [Evans et al., 2012]. This is also problematic if dosage compensation mechanisms, which equilibrate expression levels across the sexes of sex-linked genes with differing allelic counts between males and females, are disrupted during the initial stages of polyploid speciation [Orr, 1990]. Evidence of dosage compensation is

lacking in amphibians [Schmid et al., 1986, 2010, 2012], so it is possible that disruption of dosage compensation is not germane to the question of whether differences in allele content between the sex chromosomes influenced polyploidization. Either way, both of these scenarios are similar in the sense that they predict that polyploidization would be less common in lineages with substantial differences in allele content between the sex chromosomes.

As discussed above, the African clawed frogs (*Xenopus*) stand out among amphibian genera because of their high number (at least 8) of independent polyploidization events [Evans et al., 2005, 2008, 2011; Evans, 2007, 2008]. Interestingly, the sex chromosomes of *X. laevis* are essentially identical except a small sex-determining region on the W chromosome [Tymowska, 1991; Uno et al., 2013]. This also appears to be true for *X. tropicalis* [Bewick et al., 2013], and, by extension, the closely related tetraploid species in the *Silurana* group, even though sex determination in the *Silurana* group is triggered by a different gene on a different (non-homologous) chromosome from that in *X. laevis* [Yoshimoto et al., 2008; Olmstead et al., 2010; Bewick et al., 2011; Uno et al., 2013]. Furthermore, laboratory crosses indicate that tetraploidization in *Xenopus* can occur via a female triploid intermediate that carries 1 W chromosome and 2 Z chromosomes [Kobel, 1996]. This suggests that, unlike other chromosomes in the nucleus, the W chromosome needs not be duplicated by polyploidization, and thus that a copy of the W may never have segregated as an autosome after polyploidization in *Xenopus*. Together these factors may help explain the high incidence of polyploidization in this genus.

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