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Speciation Routes of Anurans

Maria Luiza Beçak

Abstract

Genome duplication was long been reported by our laboratory in Brazilian anurans belonging to the Leptodactylidae and Hylidae families. Thanks to other investigators, several polyploid species with related diploid forms were also found in diverse regions of South America, Africa and Australia. Our cytogenetic, enzymatic and molecular experiments performed in the Brazilian polyploids showed a higher level of variability resulting from tetrasomic gene expression according to the binomial equation $(p + q)^4$. These results were supported by the classical Ohno's theory of vertebrate evolution via genome duplication. Later, we suggested that the epigenetic mutations could have a role in the expansion of the tetraploid animals exploring different regions. Here, we provide an overview of the data on polyploid anurans in light of the speciation process. Some biogeographic events of Mesozoic and Cenozoic Eras probably related to anurans evolution were also focused.

Keywords: anurans evolution, genome duplication, epigenesis, species diversification, geographic speciation concept

1. Speciation rhythms: slow × fast concepts

The current neo-Darwinism concept of evolution stated that speciation is a slow accumulation of random mutations and selection of favored phenotypes. Abrupt mass extinctions of the species and the appearance of new forms occurred in the Earth due to tectonisms, volcanisms, meteors and global warming.

The neo-Darwinism theory explains that the environmental alterations are associated with DNA modifications as changes of the amount of DNA content, point mutations, differences of either gene number, order and position. Up to the classical concept, reproductive isolation

is created when allopatric populations separated by geographic barriers accumulate a differential pool of genetic mutations that prevent interbreeding. The idea that speciation is a slow process was recently supported by paleontological data indicating that some animal groups like dinosaurs and birds evolved gradually and not rapidly over long timescales [1].

Alternatively, a growing body of recent evidences suggests that new species can diversify rapidly in response to ecological alterations. An example of this hypothesis is the speciation of the killer whales demonstrated to occur in sympatric populations without geographic barriers but impelled by the search of new ecological niches [2]. Also, it has been recommended that fast speciation may also occur by epigenetic mutations affecting the mechanisms of gene regulation and being transgenerationally inherited [3–5]. Also, previous molecular results in studies with synthetic polyploids of *Brassica* suggested that fast genome changes may occur in new polyploids. This fact would favor the diversifications of both plants and animals [6].

2. Epigenetic traits in the evolution of polyploidy anurans

It is well established that each species contains a specific genome with distinct DNA sequences coding for proteins as well as an epigenome with other DNA sequences involved in gene regulation. The mechanisms of gene regulation include processes of methylation of DNA, alterations of histones by methylation or acetylation as well as noncoding DNA [7]. These mechanisms play important functions in cell differentiation during embryo development. The epigenetic traits remain in all adult life through mitosis. At gametogenesis, a new programmed epigenetic architecture is established in embryo development. Some alterations of gene expression produced by changes of these regulatory sequences without alterations of DNA sequences may create epigenetic mutations. It was suggested that this type of mutation can be transgenerationally inherited, as indicated in experiments with *Arabidopsis thaliana* [8].

In anurans, it was questioned whether epigenetic mechanisms besides polyploidy may have contributed creating diversity in this animal group [4]. This conclusion is based on about five findings reported in the tetraploid *Odontophrynus americanus*: (1) that the amount of RNA content in 4n animals is not increased [9], although they have double amount of 18S and 28S ribosomal genes [10]; (2) that a higher variability of isozymes and other proteins in 4n species is caused by the tetrasomic expression $(p + q)^4$ [11–15]. While in diploid species, the population equilibrium may be translated by the equation $(p + q)^2$, thus $p^2 + 2pq + q^2$ in tetraploid populations, it is translated by the equation $(p + q)^4$, thus $p4 + 4p^3q + 6p^2q^2 + 4pq^3 + q^4$. The geographical expansion showed by heterozygotic population is amplified, meaning increased quantitative and qualitative diversity. Although tetraploid species present double number of chromosomes, only genes of two chromosomes of the four homologous chromosomes are expressed. This fact was shown in the expression of albumin, G6PD and LDH genes in the electrophoresis analysis of tetraploid species [11]. Duplication of genes allows that although variability of the species is maintained by two genes of each allele, the two remaining homologous genes are free to mutate and eventually be positively selected leading to advantageous diversification and expansion of niches occupied by the tetraploid species [16]. These results

are coherent with the concept of evolution by gene duplication [17]; (3) that there is a lower hemoglobin content in the 4n as to the 2n animals [18]; (4) that the reduced gene expression in the 4n animals is caused by the repression of half of the rDNA cistrons [19]; (5) that there is an amphiplasty configuration characterized by diphasic stages of cell cycle revealed in the two halves of the 4n genome. It may point differences in the DNA replication time probably caused by methylation [4, 20].

Based on these data, we reasoned whether the epigenetic regulation may have contributed to the wide expansion of the polyploid species through an epigenetic transgenerational inheritance. This idea would imply that the natural selection of non-Mendelian epigenetic mutations may operate in the evolution.

3. Leptodactylids evolution and correlated geobiological events

To get a better understanding on the mechanisms that accounted for Leptodactylids evolution, we also focused on some geobiological events of the Mesozoic/Cenozoic eras that drastically altered climate and ecological environments.

The knowledge of anurans evolution is greatly impaired by the scarcity of fossil records. The extinct Ichthyostega group was found to be the most primitive ancestral of living amphibians. These animals that lived in the Devonian period of the Paleozoic era (about 400 mya) evolved to the Rachitomes in the Carboniferous period (about 300 mya). During the Triassic period of Mesozoic era, the Rachitomes evolved to the Amphibia (200 mya) [17] (**Table 1**).

Systematics and cytogenetic data reviewed by Morescalchi [21] showed that the actual anurans are reminiscent of a Leptodactylid stock in the Jurassic period (about 150 mya) and diversified since the Cretaceous before the final breakage of the supercontinent Gondwan. Probably before the Cenozoic, some forms spread across parts of Gondwanaland and perhaps oriental parts. These anurans include Leptodactylidae, Hylidae, Bufonidae and Ranidae. The diversification of these forms is also extended to the Tertiary period from Paleocene to Miocene/Pliocene (65.5–1.8 mya). The origin of the Myobatrachidae is questioned whether it is related to the Bufonidae before Gondwan breakages [21] (**Tables 1** and **2**). The family Pipidae is one of the oldest families (early Jurassic) with forms specialized in early Cretaceous [29] and probably derived from Ascaphid forms [30] or from a pro-Anura stock [31, 32]. A more recent origin (Eocene/Miocene) was indicated to Brachicephalidae, Centrolenidae and Pseudidae [21]. The genus *Odontophrynus* classified in the Leptodactylidae family [33] was recently reclassified in the family Odontophrynidae (Anura: Neobatrachia) [28].

During evolution, these anurans faced several earth alterations caused by diverse events as Pangea fragmentation, impact of meteors, volcanisms and glaciations. Some of these alterations caused mass extinctions of most plant and animal species (**Table 2**; **Figure 1A** and **B**). Among these events, Pangea splitted forming Eurasia and Gondwan supercontinents in the Jurassic period (**Figure 1A** and **B**). After new breakages in the Cretaceous (about 65 mya), Eurasia separated in North America, Europe and Asia, and Gondwan splitted in South

Era	Period	MYA	Actual families of polyploid anurans
CENOZOIC	Holocen (recent)	0,01	
	Pleistocen	1,8 − 0,01	
	Pliocen	5,3 − 1,8	
	Miocen	23,0 − 5,3	
	Oligocen	33,9 − 23,0	
	Eocen	55,8 − 33,9	
	Paleocen	65,5 − 55,8	
MESOZOIC	Cretaceous	146 − 65,5	
	Jurassic	200 − 146	Leptodactylid stock
	Triassic	251 - 200	Amphibia
PALEOZOIC	Permian	299 − 251	
	Carboniferous	359 - 299	Rachitomes
	Devonian	416 - 359	Ichthyostega
	Silurian	444 − 416	
	Ordovician	488 − 444	
	Cambrian	542 − 488	

(Actual families of polyploid anurans listed at right: Leptodactylidae, Hylidae, Bufonidae, Ranidae, Myobactrachinae, Pipidae)

Table 1. Evolution of actual polyploid anurans derived from a Leptodactylid stock: phylogenetic tree based on Ohno [17] and Morescalchi [21]; geologic periods from Teixeira et al. [22], mya = millions years ago.

Paleozoic	Mesozoic			Cenozoic					
Permian 299-251 mya	Triassic 251-300 mya	Jurassic 200-146 mya	Cretaceous 146-65,5 mya	Paleoncen 65,5-55,8 mya	Eocen 55,8-33,9 mya	Oligocen 33,9-23,0 mya	Miocen 23,0-5,3 mya	Pliocen 5,3-1,8 mya	Pleistocen 1,8-0,01 mya
Pangea supercontinent	Amphibia origen	Pangea initial breakages	Pangea final breakages						
		Leptodactylids Stock,	........*Leptodactylids diversification*						
Mass extinctions 251 mya (M or E)	210 mya (E)		*Mass extinctions – Global warming* 65 mya (M) 55 mya (GM)						
			Piratininga SP 117mya (M)	*Yucatan Mexico 65,5 mya (M)*	*Colonia SP 35 mya (M)*				
			Cerro do Jaú RGS 117 mya (M)	*Vista Alegre P 65 mya (M)*	*Global Warming 55,5 mya (GM)*				
			Vargeão SC 110 mya (M)						
							Cooling off periods	*Glaciations?*	*Glaciations*

Table 2. Evolution of Leptodactylids (based on Morescalchi) [21] and some geobiological events during Mesozoic/Cenozoic periods: continental drift [23]; glaciations and mass extinction [24, 25]; geologic times [22]; meteors [26], mya = million years ago, M = meteor, E = eruption, polyploidy was assumed to have happened in the Eocene in *Xenopus* [27] and in the *Odontophrynus* [28].
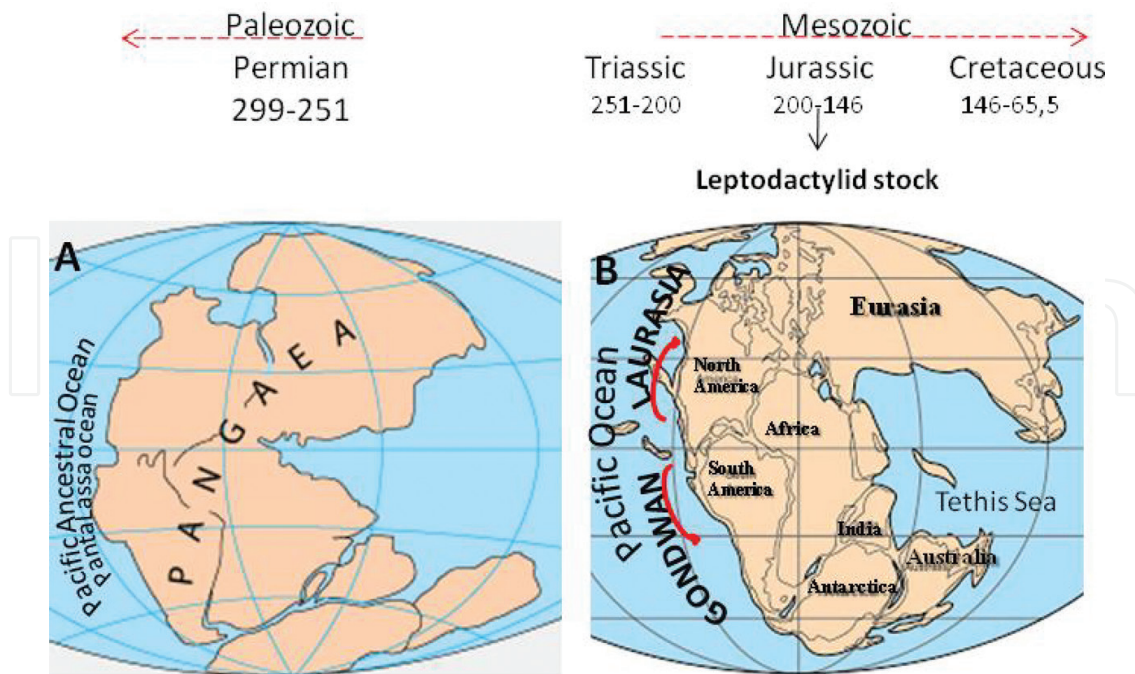
Figure 1. A schematic drawing showing the Pangea supercontinent in the Permian period (A) and its initial fragmentation in Laurasia and Gondwana during Jurassic/cretaceous (about 150 mya) followed by new fragmentation at the final of cretaceous (about 65 mya) that led to the actual configurations of the continents (B). Figures based on data by Grotzingert and Jordan [24], Gaeta Tassinari et al. [23] and geology.com/articles/supercontinent.shtml. The Leptodactylid stock occurred in the Jurassic period (about 150 mya) according to Morescalchi [21].

America, Africa, Australia and India [23] (**Figure 2**). As it is known, the movements and breakages of the ancient continents are explained by the theory of collision of tectonic plates [23, 34]. This theory confirmed the continental drift hypothesis of Wegener's (1912, 1915) on the breakages of the single earth supercontinent that led to current configuration [35].

In the case of southeastern Brazil, several meteors fell in diverse states during the Cretaceous and Cenozoic (**Table 2**; **Figure 3**). Some of these events caused mass extinction by alterations of temperature, earthquakes and volcanisms.

Another type of mass extinction occurred in the Paleocene/Eocene (about 55 mya) being caused by a global warming produced by the liberations of gas methane (**Table 2**) [25].

Several species were also eliminated by the glaciations in the Pleistocene and new species appeared. It was suggested that these glaciations could be extended to Pliocene (5.3–1.8 mya) and the cooling probably preceded the Miocene (23.0–5.3 mya). Also, it was proposed that this cooling may be related to the continental drift process [24]. Interestingly, this estimate includes the time of diversification of the Leptodactylid families from Paleocene to Miocene/Pliocene (65.5–1.8 mya).

Mass extinction also occurred in the Cretaceous probably caused by meteors (**Table 2**). In the final of Cretaceous (about 65 mya), a meteor fell on the region that today corresponds to Mexico and killed 75% of the species including the dinosaurs. It is known that the extinction of the reptiles favored mammalian expansion and evolution [26]. Another hypothesis is changing the explanation
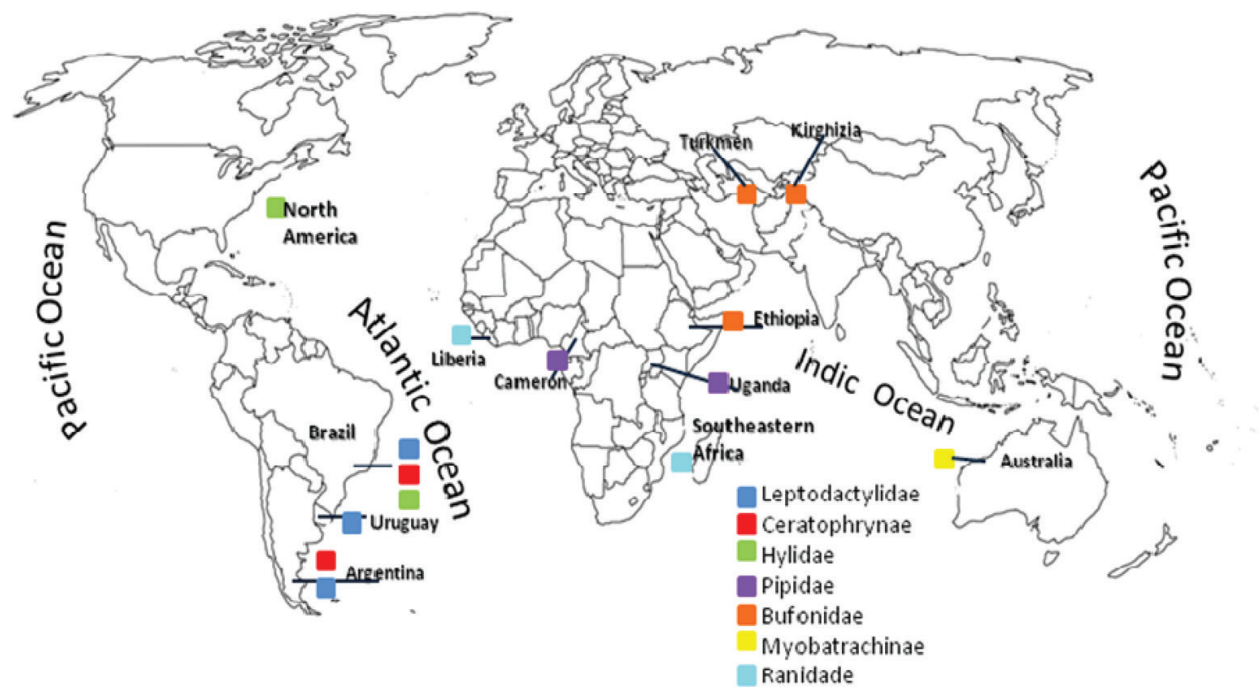
Figure 2. A schematic drawing of the present day continents with living polyploid and pos-polyploid species of anurans belonging to Gondwanian families (continental map is based on https://www.estadosecapitaisdobrasil.com/wp-content/uploads/2015/07/mapa-mundi-colorir-paises-2.pdf).

Figure 3. Localization of craters caused by meteors in southeastern Brazil during cretaceous, Paleocene and Eocene. The bigger crater in the central Brazil fell during the Triassic (based on Fairchild [26]).

that mass extinction of dinosaurs was caused only by the meteor. The data obtained in a metric called morphological disparity showed that the extinction of the carnivorous dinosaurs may also be related to the decline of the herbivorous reptiles before the striking of the meteor [36]. Also, the mass extinction of the Cretaceous was attributed to eruptions in Siberia [37].

4. Genome duplication driving Anura diversification

An extensive populational cytogenetic and molecular study was previously performed in our laboratory using Leptodactylids species derived from the Gondwanian stock. The results obtained in the genus *Odontophrynus* indicated that autopolyploidy was accounted for the diversification of the species through Brazil and other South American countries [38, 40, 51] (**Figure 4**). Other polyploid species with related 2n species were also described by several investigators in anurans from South America, Africa and Australia [41, 42] (**Tables 3** and **4**).

To verify the level of reproductive isolation between 2n and 4n species, several artificial hybridizations were performed. The mating chosen of ♀ *O. americanus* (4n = 44) × ♂ *O. cultripes* (2n = 22) and of ♀ *O. americanus* (4n = 44) × ♂ *O. americanus* (2n = 22) gave F_1 triploid hybrids (3n = 33). Some F_1 3n from ♀ *O. americanus* (4n = 44) × ♂ *O. cultripes* (2n = 22) exhibited *aneuploid* and *normal euploid* gametes. This indicated that 3n hybrids could produce descendent with higher levels of ploidy as F_2 6n animals [48, 71].
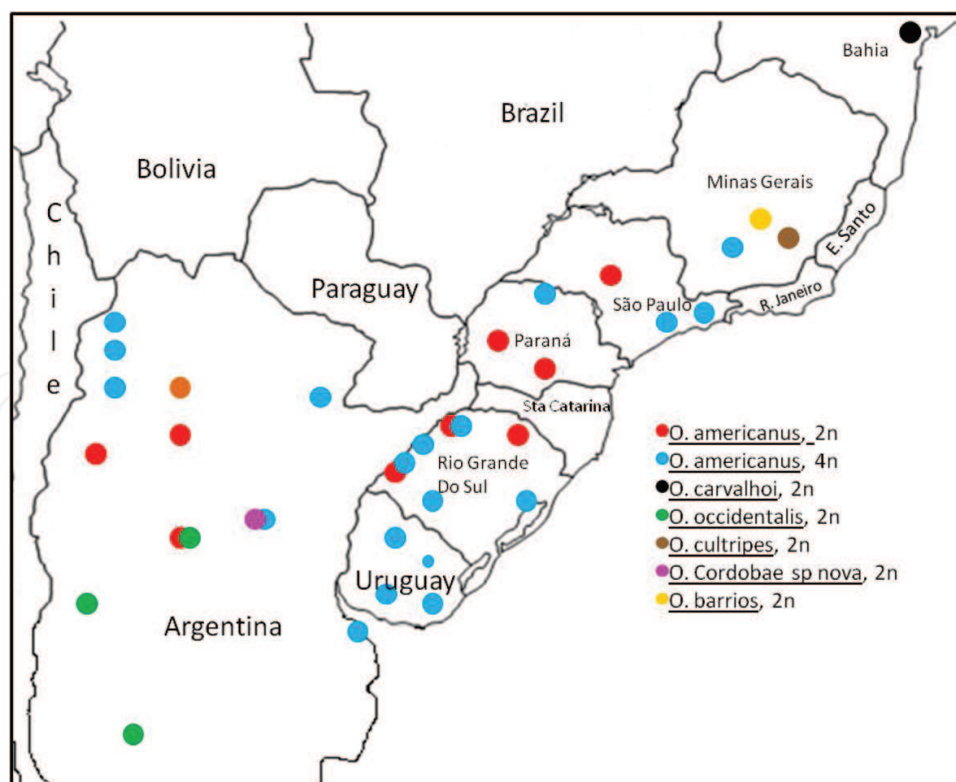

Figure 4. A schematic drawing showing the geographic distribution of diplo and tetraploid species of *O. americanus* in South American countries (see **Tables 3** and **4** for author's names) (based on Beçak [4]).

Families/species	Ploidy level	Ancestral number	References/localities
Leptodactylidae			
Odontophrynus americanus	4n = 44	n = 11	Beçak et al. [38]; Beçak [46]; Beçak et al. [39]; Beçak et al. [47, 48]; Beçak and Beçak [20]; Beçak and Kobashi [49]; Beçak [4]; Bogart [42]; Barrio and Pistol de Rubel [39]/Brazil and Argentina
Odontophrynus cordobae	4n = 44	n = 11	Martino and Sinsch [50]/Cordoba
*Ceratophrys dorsata** (= *C. aurita*)	8n = 104	n = 13	Beçak et al. [38]; Beçak [46]/Brazil
Ceratophrys ornate	8n = 104	n = 13	Bogart [42]; Barrio and Chieri [52, 53]; Bogart and Wasserman [54]/Argentina
*Eleuterodactylus binotatus**** (pos-polyploid)	2n = 22	n = 11	Beçak and Beçak [55]/Brazil
Pleurodema bibroni (*P. darwinii*)	4n = 44	n = 11	Barrio and Chieri [52, 53]/Argentina
*Pleurodema kriegi***	4n = 44	n = 11	Barrio and Chieri [52, 53]/Argentina
Pleurodema cordobae sp. nov	8n = 88	n = 11	Valetti et al. [56]; Salas et al. [57]/Argentina
Myobatrachidae			
*Neobatractus sudelli***	4n = 48	n = 12	Ptacek et al. [58]/Australia
*Neobatractus sutor***	4n = 48	n = 12	Mable and Roberts [59]/Australia
Hylidae			
Hyla versicolor+	4n = 48	n = 12	Wasserman [60]; Bogart and Wasserman [54]/North America
*Phylomedusa burmeisteri** (P. tetraploid)	4n = 52	n = 13	Beçak et al. [47]/Brazil
Pipidae			
*Xenopus vestitus***	4n = 72	n = 18	Tymowska et al. [62]/Uganda
Xenopus ruwenzoriensis++,**	6n = 108	n = 18	Tymowska and Fischberg [63]/Uganda
Xenopus sp. *wittei***	4n = 72	n = 18	Fischberg and Kobel [64]; Tinsley et al. [65]/Uganda
Xenopus amieti++	4n = 72	n = 18	Kobel et al. [45]/Cameron
Bufonidae			
Bufo danatensis	4n = 44	n = 11	Pisanetz [66]; Kawamura [93]/Turkmen
Bufo viridis	4n = 44	n = 11	Mazik et al.[67]/Kirghizia/Russia
Bufo sp.	4n = 40	n = 10	Bogart and Tandy [68]/Ethiopia
Bufo armarae	4n = 40	n = 10	Tandy et al. [70]/Ethiopia

Families/species	Ploidy level	Ancestral number	References/localities
Ranidae			
Dicroglossus occipitalis	4n = 52	n = 13	Bogart and Tandy [68]/Liberia
Pyxicephalus delalandii	4n = 52	n = 13	Bogart and Tandy [68]/Southeastern Africa

*Meiotic multivalent.

**Few or absence of multivalent.

***Meiotic multivalent rings by multiple translocations pos-polyploidy.

+Considered to be an allopolyploid that arose from hybridization between eastern and western populations of *H. chrysosceles* [44].

++It is not known whether they are auto or allopolyploid species [45] based on Beçak [4] with additions.

Table 3. Polyploid species of anurans (authors/localities are indicated).

Families/species	2n	References/localities
Leptodactylidae		
Odontophrynus americanus*	22	Beçak et al.[47, 48]; Barrio and Pistol de Rubel [39]; Bogart and Wasserman [54]; Beçak and Beçak [41]/Brazil
Odontophrynus cultripes	22	Saez and Brum-Zorrilla [69]; Beçak [46]; Beçak et al. [39]; Beçak and Beçak [71]; Beçak and Beçak [40]; Beçak and Kobashi [49]/Brazil
Odontophrynus carvalhoi	22	Beçak et al. [47]; Beçak and Beçak [41]/Brazil
Odontophrynus barrio	22	Cei et al. [72]/Argentina
Odontophrynus occidentalis	22	Saez and Brum-Zorrilla [69]; Beçak and Beçak [71]; Beçak and Beçak [41]; Ruiz et al. [73]/Argentina
Odontophrynus cordobae* *sp. nov*	22	Martino and Sinsch [50]/Argentina
Ceratophrys ornata*	26	Barrio and de Chieri [52, 53]/Argentina
Hylidae		
Phyllomedusa burmeisteri*	26	Batistic et al. [74]/Brazil
Hyla chrysoscelis*	24	Bogart and Wasserman [54]; Ralim and Selander [44]/North America
Hyla andersoni	24	Wasserman [60]/North America
Pipidae		
Xenopus laevis laevis	2n = 36	Tymowska and Fischberg [62]
X. laevis petersi	2n = 36	Tymowska and Fischberg [62]
X. laevis victorianus	2n = 36	Tymowska and Fischberg [62]
X. (laevis) borealis	2n = 36	Tymowska and Fischberg [62]
X. gilli	2n = 36	Tymowska and Fischberg [62]
X. muelleri	2n = 36	Tymowska and Fischberg [62]
X. tropicalus	2n = 20	Tymowska [75]; Tymowska and Fischberg [63]
X. fraseri	2n = 36	Tymowska and Fischberg [63]; Kobel et al. [45]

Families/species	2n	References/localities
Bufonidae		
Bufo viridis[*]	22	Mazik et al. [67]/Kirghizia—Russia
Bufo sp.	20	Bogart and Tandy [68]/Ethiopia
Ranidae		
Dicroglossus occipitalis[*]	24	Bogart and Tandy [68]/Liberia
Pyxicephalus dilalandii	24	Bogart and Tandy [68]/Southeastern Africa

Table 4. Diploid species of anurans with related polyploid species[*] (references and localities are indicated) based on Beçak [4] with additions.

Besides autopolyploidy, it was reported that centric fusions also played a role in the evolution of anurans. In Leptodactylidae, the number of chromosomes found are 2n = 18, 2n = 20, 2n = 22 and 2n = 26. In Hylidae, we found 2n = 22, 2n = 24, 2n = 26 and 2n = 30 [43]. The reduction of the chromosome number was previously explained by centric fusions by Wickbom [76]. Alterations of secondary constrictions and of *C* and *NOR* bands were also found in species of *Odontophrynus* in South America [41, 77–80].

The mechanism of genome duplication was pointed to be an important process during vertebrate diversification by creating new mutations. This idea postulated that a new gene copy could change without disturbing the function of the old one [17]. When exposed to natural selection, the modified gene copies have an opportunity to help the species to explore new niches. According to this 2R–model, vertebrates evolved through two rounds of genome duplication. The first event in chordate evolution occurred possibly in the Cambrian period of the Paleozoic era. Later, a second round might have occurred in the Devonian [17]. A third duplication was described in fish genomes in the Devonian period after the radiation of ray-finned fish (Actinopterygian and Sarcopterygia lineages) [81].

According to Clarke [82], gene duplication does not explain diversification in teleosts, which present a great diversity of body plans.

5. Conclusions

A fundamental and complex problem in the study of evolution is to understand the mechanisms leading to speciation. Considering Anura evolution, the main questions to which this discussion is addressed are as follows: (1) When polyploidy occurred in the Leptodactylids? (2) Genome duplication increases variability? (3) Is speciation of polyploid anurans orchestrated by epigenetics events? (4) Were geographic barriers required for diversification of these anurans?

About 21 living polyploid and pos-polyploid species of Anura are known. According to Morescalchi [21], the actual families of Anura descend of a same Leptodactylid stock of the Jurassic period that dispersed during Jurassic/Cretaceous periods, 'prior to the final

breaking-up of the Gondwanian supercontinent'. The diversification of these families extended to Cenozoic period (**Table 1**; **Figure 2**). It is unknown when genome duplication occurred in these anurans. The elucidation of this question is a challenging biological problem. Based on our cytogenetic results, we reasoned that the process of ploidy in *O. americanus* (4n), *Phyllomedusa burmeisteri* (4n) and *Ceratophrys dorsata* (8n) from Brazil [38, 40, 46] was a more recent event when compared with other polyploid events from Africa [4]. This conclusion is based on the fact that the whole genomes of the Brazilian anurans present multivalents at meiosis. These configurations indicate that homology is still high in each group of homologs. This observation agrees with the suggestion by Morescalchi [21] that some actual tropical species of Anura present intense karyological alterations by polyploidy correlated with the diversification process. Pos-polyploid alterations were described in the Brazilian anurans [55] and interpreted as signal of diploidizations [17]. An example of this process was reported in *Eleutherodactylus binotatus*, a diploid species (2n = 22) with a fourfold DNA content and exhibition of a dodecavalent ring in diplotene [55].

Besides *O. americanus,* other diplo and tetraploid species were studied (**Tables 3** and **4**). *Xenopus* genus of the family Pipidae, which is one of the oldest families of the Anura, probably emerged in early Jurassic [21]. *X. tropicalis* (2n = 20) is considered the most ancient extant species of this genus [83]. Experiments with *X. laevis* indicated that this species contains two pairs of loci for vitellogenin [84] and two genes for serum albumin [85]. Moreover, the finding that *X. laevis* (2n = 36) has two clusters of α- and β-globin genes while *B. tropicalis* (2n = 20) has only one cluster pointed that *X. leavis* is a probable tetraploid [86, 87]. Further studies of these genes led to the suggestion that the diversification of *X. laevis* and *X. tropicalis* happened in the Cretaceous period of the Mesozoic era at 110–120 mya. Later, it was suggested that the polyploidy of *X. laevis* happened in the Eocene period of the Cenozoic era at 40–60 mya [27]. In the case of *Odontophrynus*, it was suggested that polyploidy emerged by allopolyploidy from hybridization of ancestral diploid species during the Eocene when Earth faced several geological events [28].

Considering the level of variability of these tetraploid anurans, it was indicated that they have a tetrasomic gene expression according to the binomial equation $(p + q)^4$. This high variability probably facilitated the expansion of the 4n forms to different ecosystems.

Regarding the epigenetic events, it was reported that in the 4n specimens, gene expression is repressed by the methylation of half rDNA cistrons. This result was obtained using *in situ* hybridization experiments and NOR-banding. Epigenetic event was also observed with the detection of amphiplasty in 4n cells during mitosis. This fact indicated differences of chromatin packing between half genomes.

Yet, as to the mechanisms that cause reproductive isolation in these anurans, it remains an open question. The fact that genome duplication was a more recent event in the Brazilian anurans than in Africa indicates that polyploidy may have occurred in different times and that diversity may benefit from alterations of climate and from ecological impacts regardless of geographic barriers. This idea supports several facts as follows:

a. that polymorphisms of NORs and C-bands occur in allopatric diploid cryptic populations of *O. occidentalis* from Argentina [73];

b. that tetraploid populations which arose sympatrically from diploid ones maintain reproductive isolation bio-acoustic [54];

c. that in killer whale populations, geographic barrier is not a prerequisite for speciation [2];

d. that in members of the Poeciliidae fishes living in toxic sulphide waters speciation independent of geographical barriers [88];

e. that speciation may occur without geographic barriers [89];

f. that the hemoglobin patterns of allopatric populations of Brazilian 2n and 4n *O. americanus* indicate that speciation does not require geographic barrier [61];

g. That sympatry of both 2n and 4n specimens of *O. americanus* was reported in Santa Barbara do Sul, Brazil [90].

The argument that speciation operates without a previous geographic isolation leads us also to revive the suggestion by Lima de Faria [91]. He stated that modern species already carry molecular information of divergence from ancestral lineages before natural selection. This idea came from his analysis showing that although isolated about 50 mya in Australia, the marsupials exhibit similar phenotypic aspects to those of placental mammals. This implies that both groups evolved via similar mechanisms independent of the different habitats.

The data reviewed indicate that the anurans diversified by polyploidy and epigenetic events. Moreover, several results raise questions as to whether the process of speciation may occur without geographic barriers. These ideas sound as an alternative suggestion to the neo-Darwinism concept, establishing that the mechanism of speciation is a slow accumulation of natural mutations and selection of favored phenotypes in geographically isolated populations.

The studies here reviewed were addressed to the analysis of the role of polyploidy in anurans evolution. It is well known that polyploidy also occurs in some mammalian including human cells in organs such as in liver, heart, bone marrow and in embryos. The question that remains to be elucidated is the real role of polyploidy in mammalian cells [92].

Acknowledgements

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