Meiotic differentiation in two allopatric population groups of the tetraploid frog *Odontophrynus americanus* from Argentina

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The *Odontophrynus americanus* species complex is widely distributed in South America and is formed of diploid and tetraploid cryptic species. We studied the meiotic prophase stages of two allopatric tetraploid population groups from southeastern and northwestern Argentina. These two allopatric population groups showed a bouquet polarization in zigotene and pachytene, and in the latter stage a complete synapsis of quadrivalents and bivalents. In diakinesis, the frequencies of the different elements per cell were recorded and compared with two theoretical distributions for an autotetraploid organism. Both model tests showed the same overall results. The frequency of quadrivalents did not depart significantly from the models in southeastern populations, and must be considered autotetraploid of recent origin. However, northwestern populations have a significantly reduced number of multivalents, which can be explained by diploidization or allotetraploidy. The reduction of quadrivalents is relatively low when compared with one of the models. Additionally, different chiasmatic frequencies were observed between bivalents and quadrivalents in both population groups. The presence of extra chromosomes detected in southeastern populations and the mitotic and meiotic irregularities reported from other populations, not observed in the present study, account for a high cytological variability between populations of the tetraploid frog *Odontophrynus americanus*, which can be ascribed to polyploidy.

Key words: Anura, bivalents, chiasmata, Cycloramphidae, polyploidy, quadrivalents

INTRODUCTION

Polyploidy is a mechanism of genome evolution in which the diploid chromosome complement multiplies. Traditionally, this process has been viewed as more important in plant than in animal evolution (Muller, 1925; Otto & Whitton, 2000). However, this view has recently been criticized on several of its assumptions (Mable, 2003, 2004) and new discoveries of this process have shown that polyploidy is frequent in a wide range of animals (Otto & Whitton, 2000; Leggatt & Iwama, 2003). In spite of this, studies on animals are still scarce compared to those on plants (Mable, 2003, 2004).

Polyploidy can occur in two different ways. Autopolyploid species are characterized by possessing more than two copies of the same chromosome complement. Thus, during meiosis this leads to more than two fully homologous chromosomes that can pair either as bivalents or as multivalents. On the other hand, the allopolyploids have copies of different genomes by hybridization. This can lead to preferentially formed bivalents, with strictly homologue chromosomes, at the expense of multivalents (Jackson & Casey, 1982; Jackson & Hauber, 1994; Cuñado et al., 2005). The reduction in multivalents could be accelerated after the origin of allopolyploids by divergence from the original hybridizing genomic complements through genome reorganization and gene silence (Schmid et al., 1985; Matzke et al., 1999; Soltis & Soltis, 1999; Wolfe, 2001). Autopolyploids of ancestral origin may also reduce quadrivalent formation and the ploidy level by diploidization, but this process may be slower than in allopolyploids. Several models of multivalent formation have been described and used to test polyploidy in plants, but this approach has rarely been applied in animals (Driscoll et al., 1979; Jackson & Casey, 1982; Jackson & Hauber, 1994; Gatt et al., 1998).

In the evolution of amphibian anurans, polyploidy has played an important role. The presence of polyploid species in several non-related lineages points to the fact that this process has emerged independently and on different occasions in this group (Bogart, 1980; Mahony & Robinson, 1980; King, 1990; Ptacek et al., 1994; Mable & Roberts, 1997; Stöck et al., 2002; Holloway et al., 2006). *Odontophrynus americanus* is the first sexually reproducing vertebrate for which a polyploid origin was demonstrated (Beçak et al., 1966, 1967a,b). The high frequency of meiotic quadrivalents led to the interpretation that this species is of recent autotetraploid origin (Beçak et al., 1966; Schmid et al., 1985). Previous meiotic studies of *O. americanus* from Argentina and Uruguay have shown that the observed frequencies of multivalents are not different from those predicted for an autotetraploid organism of recent origin (Rahn & Martínez, 1983; Schmid et al., 1985). Other studies, based on low number of cells

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Table 1. Specimens, geographic origin and number of meiotic elements (total elements, quadrivalents and bivalents) and chiasmatic frequencies per cell of the *Odontophrynus americanus* specimens analysed in this study. Values are means ± 1 SD; *n* = sampling number; * indicates specimens with B chromosome.

Locality, specimen	Number of total elements	Number of quadrivalents	Number of bivalents	Chiasmata frequencies	*n*
NORTHWESTERN POPULATIONS					
San Pablo de Reyes, Jujuy, MLP DB 2198	15.84±1.77	6.13±1.82	9.68±3.54	39.71±2.36	31
San Pablo de Reyes, Jujuy, MLP DB 2199	15.47±1.59	6.50±1.61	8.93±3.18	41.47±2.30	30
San Pablo de Reyes, Jujuy, MLP DB 2200	15.50±1.53	6.50±1.53	8.93±2.99	39.57±3.43	30
La Merced, Salta, MLP DB 1643	15.83±1.72	6.17±1.72	9.67±3.45	41.22±1.70	18
Encón Grande, Salta, MLP DB 2545	14.72±1.30	7.28±1.30	7.34±2.71	42.78±1.16	32
Encón Grande, Salta, MLP DB 2547	15.33±1.52	6.67±1.52	8.67±3.03	40.63±1.65	30
Encón Grande, Salta, MLP DB 2549	15.07±1.80	6.97±1.69	8.03±3.29	42.40±1.33	30
Encón Grande, Salta, MLP DB 2552	15.47±1.55	6.47±1.57	8.93±3.10	41.53±2.15	30
Encón Grande, Salta, MLP DB 2561	14.97±1.43	7.03±1.43	7.93±2.85	42.53±1.20	30
Encón Grande, Salta, MLP DB 2579	15.20±1.49	6.73±1.55	8.40±2.99	38.90±1.83	30
Encón Grande, Salta, MLP DB 2580	15.11±1.64	6.86±1.65	8.23±3.28	41.43±1.72	35
Encón Grande, Salta, MLP DB 2581	15.08±1.55	6.92±1.55	8.15±3.09	41.08±1.52	26
Encón Grande, Salta, MLP DB 2582	14.73±1.62	7.27±1.62	7.47±3.23	42.10±1.35	30
Salta, Salta, MLP 3716	15.17±1.88	6.83±1.88	8.27±3.70	41.93±1.57	30
Salta, Salta, MLP 3890	15.59±1.78	6.41±1.78	9.07±3.70	42.62±1.18	29
El Mojón, Catamarca, MLP DB 2660	15.10±1.59	6.90±1.63	8.20±3.25	41.00±1.31	30
El Mojón, Catamarca, MLP DB 2745	15.27±1.20	6.73±1.20	8.40±2.54	42.00±1.26	30
El Mojón, Catamarca, MLP DB 2746	15.40±1.61	6.60±1.61	8.60±3.20	41.60±1.30	30
Rosario, Catamarca, MLP DB 2670	15.00±1.47	7.00±1.47	8.00±2.95	42.63±1.21	24
SOUTHEASTERN POPULATIONS					
Loreto, Corrientes, MLP DB 2311*	14.13±1.53	7.87±1.53	6.27±3.05	42.40±1.54	30
Vera, Santa Fe, MLP DB 2718	14.89±1.59	7.43±1.95	7.32±3.13	40.32±1.87	28
Mojones, Entre Ríos, MLP 3715	15.07±1.74	6.93±1.74	8.13±3.48	42.47±1.41	30
Tres Bocas, Entre Ríos, MLP 3712	14.43±1.36	7.57±1.36	6.87±2.71	41.93±1.82	30
Paraná, Entre Ríos, MLP DB 2109	14.45±1.41	7.55±1.41	6.90±2.82	41.58±1.69	31
Pilar, Buenos Aires, MLP 3888	14.10±1.45	7.93±1.44	6.17±2.88	43.10±0.89	30
Pilar, Buenos Aires, MLP 3931	14.80±1.27	7.20±1.27	7.47±2.46	41.50±1.74	30
Magdalena, Buenos Aires, MLP 3922	14.17±1.42	7.83±1.42	6.33±2.83	42.90±0.99	30
Magdalena, Buenos Aires, MLP 3923	14.57±1.65	7.30±1.68	7.27±3.30	42.33±1.15	30
Magdalena, Buenos Aires, MLP 3924	14.56±1.48	7.40±1.48	7.13±2.86	42.53±0.97	30
Magdalena, Buenos Aires, MLP 3925*	15.53±1.78	6.43±1.83	8.93±3.42	42.10±1.65	30
Magdalena, Buenos Aires, MLP 3980	14.40±1.61	7.60±1.61	6.80±3.22	43.23±0.97	30
Magdalena, Buenos Aires, MLP 4555	14.87±1.46	7.13±1.46	7.73±2.91	42.47±1.17	30
Punta Indio, Buenos Aires, MLP 3713	14.06±1.57	7.94±1.57	6.13±3.14	42.65±1.31	31
Mar Chiquita, Buenos Aires, MLP DB 3489	14.80±2.06	7.17±2.04	7.60±4.12	42.03±1.30	30

from specimens from Brazil, revealed a higher number of multivalents (Beçak et al., 1967a).

We studied and compared the synaptic behaviour and multivalent formation in two allopatric population groups of the tetraploid frog *Odontophrynus americanus* from Argentina. These groups possibly constitute two different tetraploid species (for review see Rosset et al., 2006). Moreover, we tested the hypothesis of the origin of polyploidy using two theoretical distributions for an autotetraploid organism with 2n=4X=44 (Sybenga, 1975; Jackson & Casey, 1982). The presence of two different extra chromosomes was detected and meiotic behaviour was also analysed in order to account for the variability of polyploidy in this species complex.

MATERIALS AND METHODS

We analysed the meiosis of 34 tetraploid males of *Odontophrynus americanus* from two Argentinean allopatric population groups. The southeastern group (population group 1 of Rosset et al., 2006) includes samples from Buenos Aires, Entre Ríos, Corrientes and Santa Fe provinces, while the northwestern group (population group 3 of Rosset et al., 2006) includes samples from Salta, Jujuy and Catamarca provinces (Table 1, Fig. 1). A review of the karyotypic data of the *O. americanus* species complex has been published previously (Rosset et al., 2006). Chromosomal meiotic preparations were obtained following the standard methodology (Schmid, 1978). Staining

Fig. 1. Map showing the study localities of the tetraploid frog *Odontophrynus americanus*. Localities for the northwestern group (triangles) are: 1) San Pablo de Reyes, 2) La Merced, 3) Encón Grande, 4) Salta, 5) El Mojón, 6) Rosario; localities for the southeastern group (squares) are: 1) Loreto, 2) Vera, 3) Mojones, 4) Tres Bocas, 5) Paraná, 6) Pilar, 7) Magdalena, 8) Punta Indio, 9) Mar Chiquita.

was carried out with 10% buffered Giemsa and observed using a photomicroscope (OLYMPUS BX50F-3) and a videocamera (SONY ExwaveHAD). We analysed the early stages of meiosis. We used X (basic chromosome number), n (gametic chromosome number) and 2n (somatic chromosome number), as suggested by White (1954). For each individual we counted the number and type of elements, discriminating among univalents (Is), bivalents (IIs), trivalents (IIIs) and quadrivalents (IVs), and determined the frequency of chiasmata per cell at diakinesis. For some of them, metaphase II chromosomes were also counted. The statistical analyses were performed using STATISTICA 6.0 software.

We used models to test autopolyploidy in both the northwestern and southeastern population groups of *Odontophrynus americanus* following studies on other polyploid species (Jackson & Casey, 1982; Jackson & Hauber, 1994; Gatt et al., 1998). To calculate the expected number of IVs per cell for an autotetraploid organism with 2n=44, X=11 chromosomes, we considered that each chromosome group was completely homologous and that pairing can be initiated at two independent sites at the end of chromosomes. This assumption gives a ratio of two IVs to one set of two IIs in every homologous group (Sybenga, 1975). Following this theoretical model, the total expected frequency of IVs per cell was calculated using the formula $f(x) = p^r . q^{n-r} . {}_nC_r$, where p = probability of IV formation in one homologous group (2/3); q = probability of II formation (1/3); n = total number of homologous groups (11); r = possible number of IVs (0–11); $n–r$ = possible number of homologous IIs (0–11); and ${}_nC_r$ = number of possible combinations in the eleven homologous groups.

The model of Jackson & Casey (1982), together with the pairing probabilities, incorporates the chiasma frequency. Chain and ring elements are discriminated. The maximum chiasma frequency was calculated according to a model with two chiasmata per II or four per IV, which is in fact observed to be the maximum in *Odontophrynus americanus*. The ratio between maximum and observed chiasma frequency was calculated and incorporated into the equations. The observed frequency of IIs and IVs was obtained by grouping all cells. Both models were tested with χ^2 tests. Frequencies of quadrivalents lower than one were pooled. Analyses of variance (ANOVA) were conducted to determine whether mean cell quadrivalent frequencies varied significantly among different individuals. Homogeneity of variances was tested with Bartlett's test (Sokal & Rohlf, 1998).

RESULTS

All normal meiotic stages were observed in cytological preparations. Pachytene and zygotene were very frequent in some individuals (Fig. 2). A very marked and persistent bouquet polarization was observed in these stages, with telomeres grouped in a restricted region of the nucleus (Fig. 2a,b). In general, zygotenes had notable differences in the degree of condensation and pairing between telomeres and the internal regions of the chromosomes. While telomeric regions were paired and condensed, internal regions were unpaired and highly decondensed indicating early stages of synapsis (Fig. 2a,b). Also, the observation of internal asynaptic loops in IIs and IVs in late zygotene and pachytene (Fig. 2c–g) could indicate that the initiation of chromosome pairing was terminal and bidirectional. Other methods, like electron microscopy of the synaptonemical complex and FISH with telomeric probes, are necessary to confirm all these conclusions.

In general, full synapsis in IIs and IVs was observed in pachytene. Short internal asynaptic regions, in the joining of four elements, were observed in some IVs (Fig. 2f,g). At low frequency, the presence of relatively long asynaptic regions was observed in these elements as well as in the joining of unpaired regions among IVs. Also at low frequency, delay in the pairing of complete chromosome arms was observed (Fig. 2d,e). In some preparations with intense staining, the presence of a nucleolus in zygotene was observed by the end of pachytene, when it probably disintegrated. Diplotene was not frequently observed, indicating its short persistence in the cellular cycle.

Diakinesis was a frequent stage, and IIs and IVs were very frequent (Fig. 3a–e); Is and IIIs were not frequent

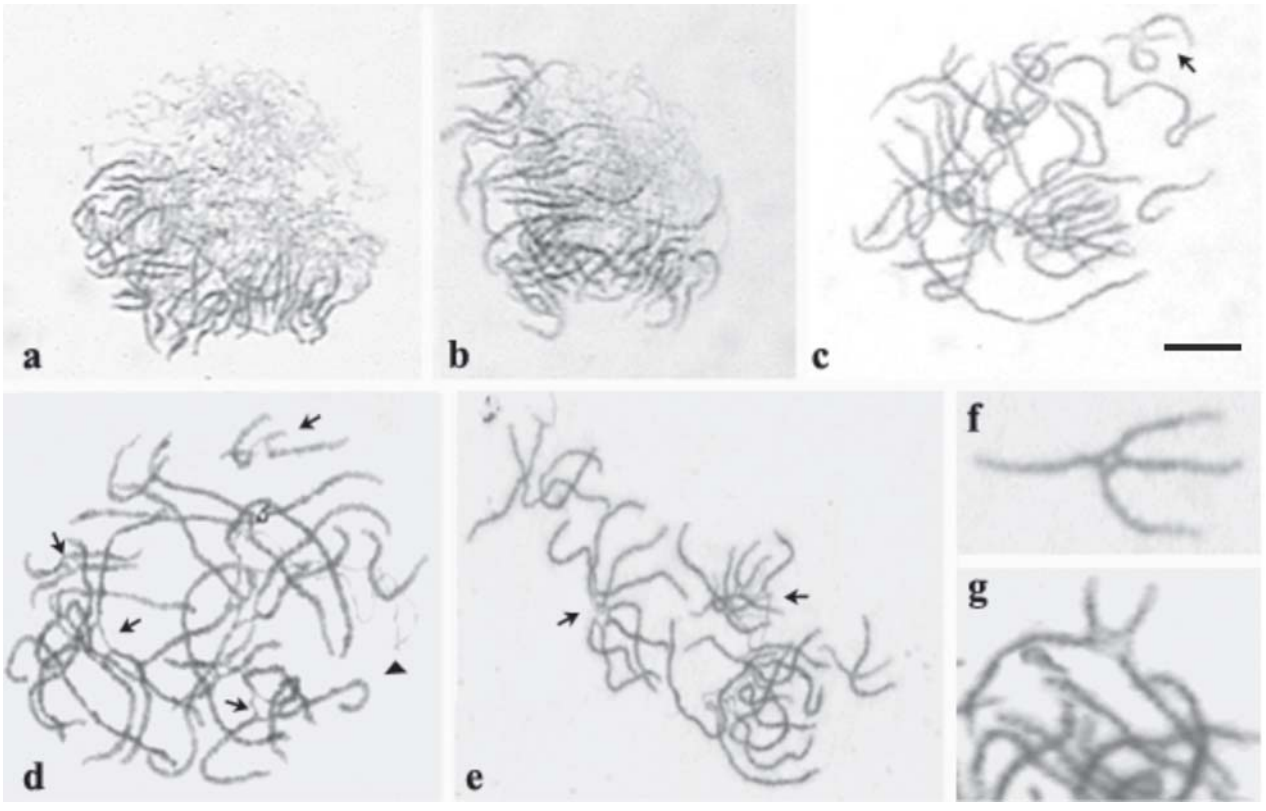

Fig. 2. Meiotic prophase of *Odontophrynus americanus*. a–b) Zygotene with a bouquet polarization, all telomeres are paired and clustered in a restricted region of the nucleus. Internal regions are unpaired and projected to the opposite cellular pole; c) pachytene with almost complete pairing of a quadrivalent (arrow); d–e) pachytene where unpaired internal regions are observed in some quadrivalents (arrows), as well as pairing delay in large chromosome regions (triangle); f–g) selected quadrivalents. The bar corresponds to 10 μm.

(Fig. 3f,g). Out of 1007 diakinesis cells, 18 (1.79%) presented Is originating from some of the quartets. In 15 cells one or two IIIs and one or two Is were observed; and three cells showed two Is caused by chiasma failure in one II. Thus, in 83.33% of cases, the homologues of Is were associated with IIIs. The presence of close IIIs, which would suggest synapsis among the six arms of three homologues, was not observed at this stage either. Multivalents with more than four chromosomes were rare. Hexavalents and octavalents were observed in some cells of one individual from Salta, which may be the result of chromosome rearrangement (Fig. 3h). In another individual, one cell in diakinesis possessed octavalents and multivalents of lower order, probably produced by a non-reduced complement (Fig. 3i). Chiasmata were usually terminal.

The mean frequency of IVs per cell at diakinesis varied from 6.13±1.82 in one specimen from Jujuy, to 7.94±1.57 in one specimen from Buenos Aires (Table 1). ANOVA showed that the difference in the number of IVs among individuals without discriminating their geographic origin was statistically significant (F=2.82; df=1,33; P<0.0001). Bartlett's test for homogeneity of variances was not statistically significant (χ^2=24.29; df=33; P=0.86). When geographic origin was taken into account, there were no significant differences between individuals from Salta, Jujuy and Catamarca (F=1.10; df=1,18; P=0.35). Specimens from these provinces came from northwestern Argentina and were grouped for later analyses of the frequency of IVs. In contrast, significant differences were observed between individuals from southeastern populations (F=2.02; df=1,14; P=0.016). This was due to the presence of one individual from Buenos Aires (Tukey test) carrying one extra chromosome (see below), which presented a reduced frequency of IVs (Table 1). When this individual was excluded from the analysis, no significant differences were detected among samples from Buenos Aires, Corrientes, Entre Ríos and Santa Fe (F=1.24; df=1,13; P=0.25). The mean frequency of IVs per cell was significantly different when northwestern and southeastern samples were compared, independently of whether the specimen carrying an extra chromosome was included (F=40.21; df=1,1; P>0.0001) or not (F=48.36; df=1,1; P>0.0001).

The distribution of IVs per cell (n=1007) shows a peak at seven, with six and eight being the next largest categories (data not shown). This distribution has a significantly lower frequency of IVs (χ^2=50.56; df=10; P<0.001) than expected for a theoretical distribution calculated for an organism with 2n=4X=44 (Fig. 4a). When the samples were separated by geographic origin, the frequency of IVs per cell from northwestern individuals (n=555) was significantly different from the expected one (χ^2=83.20; df=9; P<0.001); again a reduction in the number of IVs, but more markedly, was observed, which was also present in the analysis of the whole sample, with two peaks at six

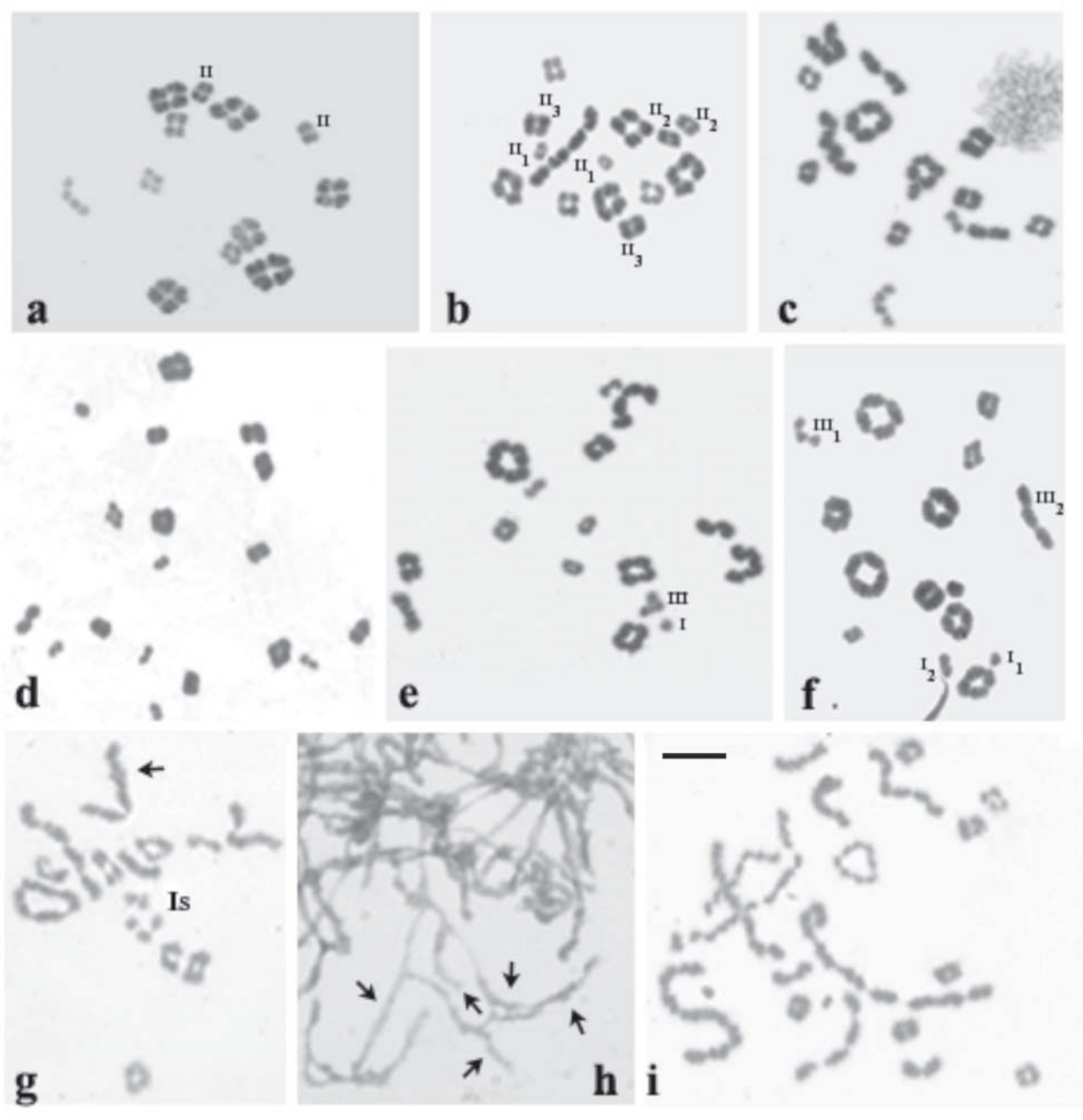

Fig. 3. Diakinesis cells of *Odontophrynus americanus* with a) 10 IVs and 2 IIs; b) 8 IVs and 6 IIs; c) 7 IVs and 8 IIs; d) 4 IVs and 14 IIs. Note that all IIs have an homologous counterpart of similar size. Cells with e) one III and one I; f) 2 IIIs and 2 two Is; g) cell from one individual showing hexavalent (arrow) and five Is in close association; h) zigonete detail of the same individual where more than five arms (arrows) are associated in one multivalent; i) non reduced diakinesis. The bar corresponds to 10 μm.

and seven (Fig. 4b). The distribution of IVs per cell in individuals from southeastern populations (n=452) has two major peaks at seven and eight (Fig. 4c), and these frequencies are not different from those expected for an autotetraploid with 2n=4X=44 chromosomes (χ^2=3.12; df=9; P=0.96).

Only one distal chiasma by chromosome arm was observed. The mean chiasma frequency per cell varied from 38.9±1.83 in one specimen from Salta to 43.23±0.97 in one specimen from Buenos Aires (Table 1). To test the model of Jackson & Casey (1982), cells from individuals from northwestern and southeastern populations were grouped. The mean chiasmata frequencies were 41.55 and 42.23 for each group, respectively. With only one chiasma per arm, the maximum number of chiasmata per cell was 44. This resulted in P=0.94 for the northwestern sample and P=0.96 for the southeastern one, with Q=1–P (see Jackson & Casey, 1982). The observed and expected frequencies are shown in Table 2. The total frequency of configurations showed the same tendency as in the previous model. The frequencies of IVs and IIs in the southeastern sample were not significantly different from those expected for an autotetraploid organism of recent origin. However, a lower number of IVs was observed in the northwestern sample, and the difference was significant. When chain and ring configurations were

Table 2. Analysis of observed and expected numbers of meiotic configurations in *Odontophrynus americanus* following the Jackson & Casey's model in southeastern (SE) and northwestern (NW) populations; c.c. = equations including the correction coefficients suggested by Jackson and Hauber (1982). oIV = ring quadrivalents with four chiasmata; cIV = chain quadrivalents with three chiasmata; oII = ring bivalents with two chiasmata; cII = chain bivalents with one chiasma.

Sample		*n*	IV	II	*P*	oIV	cIV	oII	cII	*P*
SE	Obs.	452	3341	3254		3034	307	2747	507	
	Exp.		3284.3	3334.98	NS	2813.096	471.27	3048.74	286.21	<0.0001
	c.c.					2812.70	471.20	3048.31	327.83	<0.0001
NW	Obs.	555	3744	4693		3246	498	3789	904	
	Exp.		3992.75	4121.24	<0.0001	3196.78	795.93	3594.76	526.44	<0.0001
	c.c.					3195.37	795.58	3593.18	634.92	<0.0001

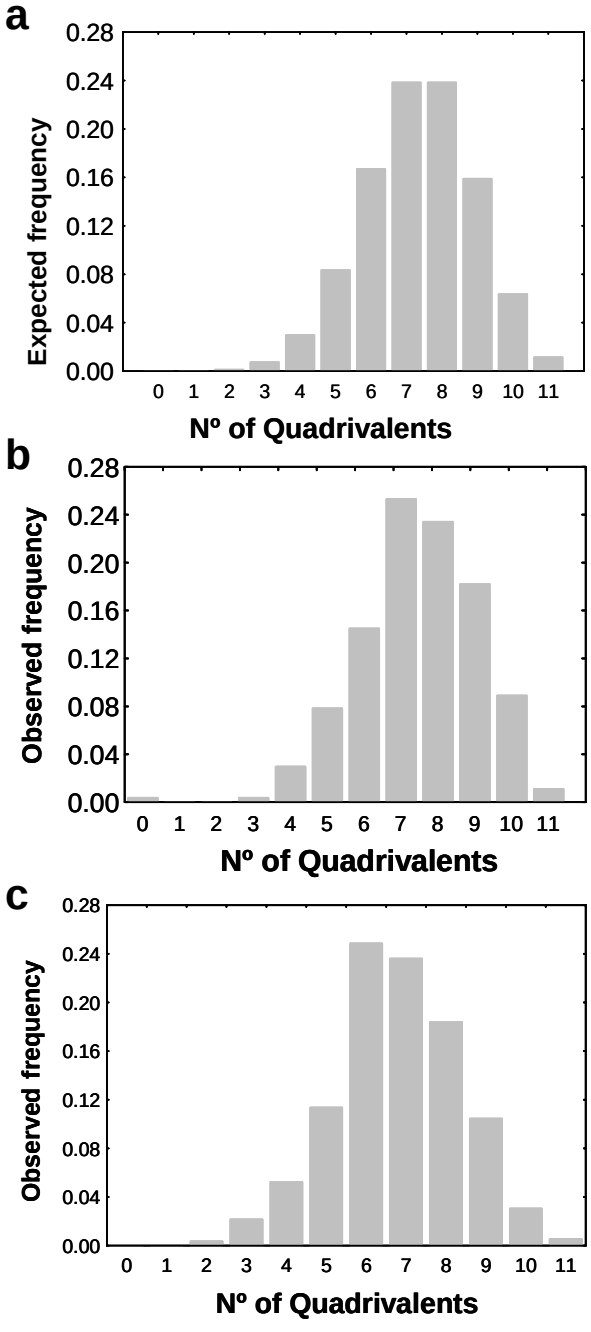

Fig. 4. Relative frequency of quadrivalents of *Odontophrynus americanus* in diakinesis. a) Theoretical; b) observed in southeastern populations; c) observed in northwestern populations.

considered, both samples departed significantly from the model, even when a correction factor suggested by Jackson & Hauber (1982) was incorporated into the calculations (Table 2). In both samples there was an excess of four-chiasmata IVs and a deficiency of three-chiasmata IVs. In IIs, an excess of one-chiasmata elements was observed (Table 2).

The frequency of IVs per cell was correlated with the frequency of chiasmata, and the regression model was significant ($P<0.001$). However, the coefficient of regression was low ($r^2=0.09$) because of a great dispersion of data. Low and high chiasmata frequencies produced chain and ring configurations respectively. Both IIs and IVs appear to be affected by the chiasma frequency, but not in a simple way (Table 3). When the regressions of chain and ring IVs and IIs were calculated separately in relation to chiasma frequency, the fittest response to the decrease in chiasma frequency was that of IIs (Table 3).

We found two individuals with extra chromosomes, which differed in morphology, size and meiotic behavior. The specimen from Corrientes (MLP DB 2311) had a micro-chromosome not similar to any other in the complement (Rosset et al., 2006), and was observed to have a high frequency in diakinesis (Fig. 5a). Presence of this B-chromosome was observed in 22 of 30 cells analysed, showing a high incidence at this stage (73.33%). Its frequency in metaphase II was not different ($\chi^2=0.053$; $P=0.01$) from that expected for a normal segregation given its observed frequency in diakinesis, suggesting that this extra chromosome is a stable element in the first meiotic division. This extra chromosome was observed in mitosis, and a precocious separation of their sister chromatids was previously described (Rosset et al., 2006). The extra chromosome in the individual from Buenos Aires (MLP 3925) was medium sized, similar to pair 10 of the standard complement (Fig. 5b–d). It was observed in a low number of cells in diakinesis and associated as trivalent in one cell and as univalent in another (Fig. 5b). In metaphase II (Fig. 5c), it can present one or two copies. This extra chromosome is reported here for the first time, and in mitosis (Fig. 5d) showed a high incidence and variable number per cell (up to four).

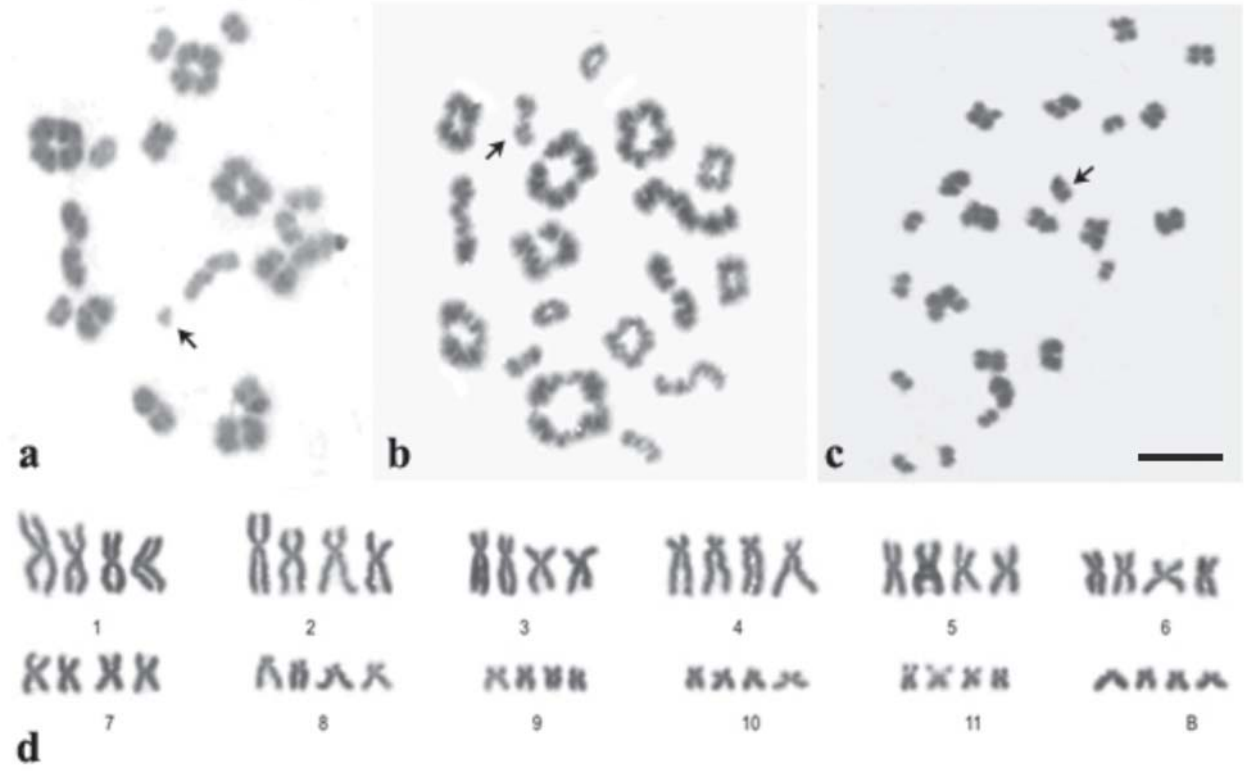

Fig. 5. Meiotic cells of *Odontophrynus americanus* carrying the B chromosomes (arrows). a) Diakinesis of the individual from Corrientes; b) diakinesis and c) metaphase II of the individual from Buenos Aires; d) karyotype showing four extra chromosomes of the latter individual. The bar corresponds to 10 μm.

DISCUSSION

Although not in all individuals, the classical stages of meiosis with complete pairing of IIs and IVs in pachytene were observed in samples from the different regions of Argentina studied here. These results agree with previous reports from Brazil (Mendes Carneiro, 1975), but are not in accord with other published data from the same region, where complete pairing of elements was not observed (Beçak et al., 1967a). All of these studies reported a bouquet-like configuration in zygotene with a great delay in the internal pairing. This meiotic behavior could favour the correct joint of the four chromosomes at their terminal region, and would facilitate the homologous synapsis. Additionally, the absence of univalents and trivalents in pachytene and their low frequency at diakinesis in *Odontophrynus americanus*, as reported for other polyploid species, indicates the presence of some regulatory mechanism that prevents associations of this kind (Mahony & Robinson, 1980; Jackson & Casey, 1982; Rahn & Martínez, 1983; Jones & Vincent, 1994).

In polyploid amphibian anurans normal multivalent formation has been observed in several species (Beçak et al., 1967a, 1970; Barrio & Rinaldi de Chieri, 1970; Bogart & Wasserman, 1972; Mahony & Robinson, 1980; Schmid et al., 1985; Tymowska, 1991; Haddad et al., 1994). However, none of these studies tested the polyploid models. The frequency of total IVs in the southeastern populations of *Odontophrynus americanus* is in accordance with data obtained in previous studies on populations from Buenos Aires and Uruguay (Rahn & Martínez, 1983). They also agree with the theoretical distribution for an autotetraploid organism with 2n=4X=44, whether the chiasma frequency is considered or not. However, the

Table 3. Linear regression equations and ANOVAs between the frequencies of quadrivalents (IV), ring quadrivalents (Ring IV), ring bivalents (Ring II), chain quadrivalents (Chain IV) and chain bivalents (Chain II) per cell and the chiasma frequency (Ch.) in all cells of the *Odontophrynus americanus*.

Relationship	Linear regression equation	F	R	*P*
IV vs Ch.	= –3.65 + 0.26 Ch.	94.49	0.29	<0.0001
Ring IV vs Ch.	= –14.11 + 0.49 Ch.	358.45	0.51	<0.0001
Chain IV vs Ch.	= 10.46–0.23 Ch.	298.92	0.48	<0.0001
Ring II vs Ch.	= –3.8 + 0.25 Ch.	23.93	0.15	<0.0001
Chain II vs Ch.	= 31.66 – 0.72 Ch.	2151.40	0.83	<0.0001

numbers of observed ring and chain configurations depart significantly from Jackson & Casey's model. As in the northwestern populations, higher frequencies of chain IIs and lower frequencies of chain IVs were observed. This lower chiasma frequency in IIs than in IVs probably reflects a non-random chiasma distribution (Jackson & Casey, 1982).

On the other hand, the northwestern populations showed a lower frequency of IVs than the southeastern populations and also than both theoretical models. This indicates a possible origin by both allopolyploidy or autopolyploidy with posterior diploidization of the northwestern populations (see below). In the model that considered only synaptic pairing but frequency of IVs in each cell, low curve displacement is observed in the northwestern sample. When the data were compared to Jackson & Casey's model there was a marked deficit in observed IVs. A higher than expected frequency of IIs has been reported for several allopolyploids and some autopolyploid species (Jackson & Casey, 1982; Orellana & Santos, 1985; Jackson & Hauber, 1994; Gatt et al., 1998; Qu et al., 1998).

Mechanisms controlling formation of IIs in polyploidy have been extensively discussed in the literature (Jackson & Casey, 1982; Orellana & Santos, 1985; Jackson & Hauber, 1994; Gatt et al., 1998; Qu et al., 1998; Cuñado et al., 2005). They may act at zygotene by preferential pairing between two specific chromosomes in the quartets, but also at pachytene by preventing chiasma formation in homologous (versus identical) associations or by reducing chiasma frequency in configurations of IVs (Orellana & Santos, 1985; Cuñado et al., 2005). Previous studies in *Odontophrynus* have indicated that a high positive correlation between mean chiasma frequency and multivalent frequency exists (Rahn & Martínez, 1983). Conversely, we observed a low positive correlation between these variables. The regulation of IV formation by reducing chiasma frequency may lead to a high frequency of aneuploid gametes due to the presence of Is. Interestingly, in Jackson & Casey's (1982) model, a decrease in chiasma frequency produced a great increase in I formation. In this study, we found seven of eleven cells with Is with a chiasma frequency lower that the mean of all individuals. Our results show that the frequency of Is belonging to IIIs more than doubles that of those pertaining to IIs, which suggests a major tendency of multivalents to produce meiotic disturbances.

Chromosome differentiation may play an important role in the preferential pairing behaviour of polyploid species (Benavente & Orellana, 1991). Studies with chromosome banding in these species have shown chromosome rearrangements and variation in heterochromatin content for a low number of homologous groups (Schmid et al., 1985; Beçak & Beçak, 1998). In this sense, it has been proposed that small chromosome changes can be sufficient to initiate diploidization (Schmid et al., 1985; Beçak & Beçak, 1998) promoting only II formation. In this context, our results from the northwestern populations of *Odontophrynus americanus* could be alternatively explained by the hypothesis of an autopolyploid origin and posterior diploidization. However, in some polyploids, it has been observed that meiotic behaviour is independent of the degree of divergence displayed by the genomes involved (Qu et al., 1998; Cuñado et al., 2005). Furthermore, it has been postulated that homology is not the only factor that determines multivalent formation (Mable, 2004).

In summary, based on the hypothesis of autopolyploid origin, differentiation between both population groups can be explained by several hypotheses, one being differential rates and maintenance of mutations between populations, and another the possible independent autopolyploid origins of these populations, the northwestern ones being a putative older lineage which accumulated more chromosome mutations among homologous groups. The wide distribution and interdigitation of the diploid and tetraploid populations point to the multiple origins of the polyploid in the *Odontophrynus americanus* species complex. Moreover, these disjunct tetraploid populations are in close association with different diploid species, and diploid species overlap in their geographical ranges (Rosset et al., 2006). Thus, an allopoliploid origin by hybridization between diploid species with high genome homology may be another hypothesis to consider, and the hybrid genome of highly related taxa may promote a high frequency of multivalent formation like the one observed in northwestern samples. Molecular studies are necessary to determine whether the origin is multiple or not.

Generally, it has been proposed that polyploidy plays a fundamental role in providing genetic variability (Soltis & Soltis, 1995, 1999; Beçak & Kobashi, 2004). Available cytogenetic data for *Odontophrynus americanus* have shown variability between populations in C and R-banding patterns and in the position and composition of secondary constrictions (Ruiz et al., 1981; Schmid et al., 1985; Cortadas & Ruiz, 1988; Schmid et al., 2003; Rosset et al., 2006). Moreover, some tetraploid specimens from Brazil exhibit asynchrony in the cell division cycle, and other genome instabilities (Beçak & Beçak, 1998) not observed in specimens from other regions (Rahn & Martínez, 1983; present study). These Brazilian populations appeared not to possess pachytene and B chromosomes. The presence of extra chromosomes was recorded only in individuals from the southeastern part of the distribution range of the genus (Rosset et al., 2006; present study). All these data showing a high variability in chromosome structure and behaviour between different populations of *O. americanus* are indicative of the independent evolution of these populations.

Only a few species with B chromosomes have been reported in Anura, and in general their presence has been detected in a few specimens of the samples studied (for a review see Green, 2004). However, these extra chromosomes present a considerable diversity, varying in number, size and morphology (Green, 2004). Similarly to other anurans, the frequency of extra chromosomes in populations of *Odontophrynus americanus* is low (Rosset et al., 2006; present study). The two different extra chromosomes studied here had different morphology, size and meiotic behaviour. The one from Buenos Aires appears to be similar to other chromosomes of the stand-

ard complement and would have originated from a recent polysomy. It was present in a low number of cells and the pairing with a bivalent in a trivalent configuration would indicate some homology with the A complement. The establishment of a B chromosome system requires genetic isolation as a first step, whereby homologous or homeologous pairing with the A complement is prevented (Camacho et al., 2000). This genetic isolation has not definitely been acquired in this B chromosome.

In contrast, the micro-chromosome B from Corrientes showed normal segregation at meiosis and thus can be efficiently transmitted to the next generation. However, the precocious separation of its sister chromatids during mitosis suggests some possible defects in the somatic divisions. The origin of this B chromosome is unknown. It may be the result of an old polysomic event, emerging as a byproduct of chromosome rearrangements, as was proposed for other organisms by Camacho et al. (2000). This suggests that the populations from southeastern Argentina present a different B chromosome system. However, further studies on samples from these localities are necessary to assess the morphology, frequency and behaviour of B chromosomes in *Odontophrynus americanus*.

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REFERENCES

Barrio, A. & Rinaldi de Chieri, P. (1970). Relaciones cariosistemáticas de los Ceratophryidae de la Argentina (Amphibia, Anura). *Physis* 30, 321–329.

Beçak, M.L. & Beçak, W. (1998). Evolution by polyploidy in Amphibia: new insights. *Cytogenetics and Cell Genetics* 80, 28–33.

Beçak, M.L. & Kobashi, L.S. (2004). Evolution by polyploidy and gene regulation in Anura. *Genetics and Molecular Research* 3, 195–212.

Beçak, M.L., Beçak, W. & Rabello, M.N. (1966). Cytological evidence of constant tetraploidy in the bisexual South American frog *Odontophrynus americanus*. *Chromosoma* 19, 188–193.

Beçak, M.L., Beçak, W. & Rabello, M.N. (1967a). Further studies on polyploid amphibians (Cerarophrydidae) I. Mitotic and meiotic aspects. *Chromosoma* 22, 192–201.

Beçak, W., Beçak, M.L., Lavalle, D. & Schreiber, G. (1967b). Further studies on polyploid amphibians (Ceratophrydidae) II. DNA content and nuclear volume. *Chromosoma* 23, 14–23.

Beçak, M.L., Denaro, L. & Beçak, W. (1970). Polyploidy and mechanisms of karyotypic diversification in Amphibia. *Cytogenetics* 9, 225–238.

Benavente, E. & Orellana, J. (1991). Chromosome differentiation and pairing behavior of polyploids: an assessment on preferential metaphase I associations in colchicine-induced autotetraploid hybrids within the genus *Secale*. *Genetics* 128, 433–442.

Bogart, J.P. (1980). Evolutionary implications of polyploidy in amphibians and reptiles. In *Polyploidy. Biological Relevance*, 341–378. Lewis, W.H. (ed.). New York and London: Plenum Press.

Bogart, J.P. & Wasserman, A.O. (1972). Diploid-polyploid cryptic species pairs: a possible clue to evolution by polyploidization in anuran amphibians. *Cytogenetics* 11, 7–24.

Camacho, J.P.M., Sharbel, T.F. & Beukeboom, L.W. (2000). B-chromosome evolution. *Philosophical Transactions of the Royal Society* 355, 163–178.

Cortadas, J. & Ruiz, I.R.G. (1988). The organization of ribosomal genes in diploid and tetraploid species of the genus *Odontophrynus* (Amphibia, Anura). *Chromosoma* 96, 437–442.

Cuñado, N., Blazquez, S., Melchor, L., Pradillo, M. & Santos, J.L. (2005). Understanding the cytological diploidization mechanism of polyploid wild wheats. *Cytogenetic and Genome Research* 109, 205–209.

Driscoll, C.J., Bielig, L.M. & Darvey, N.L. (1979). An analysis of frequencies of chromosome configuration in wheat and wheat hybrids. *Genetics* 91, 755–767.

Gatt, M., Ding, H., Hammett, K. & Murray, B. (1998). Polyploidy and evolution in wild and cultivated *Dahlia* species. *Annals of Botany* 81, 647–656.

Green, D.M. (2004). Structure and evolution of B chromosomes in amphibians. *Cytogenetic and Genome Research* 106, 235–242.

Haddad, C.F.B., Pombal Jr, J.P. & Batistic, R.F. (1994). Natural hybridization between diploid and tetraploid species of leaf-frogs, genus *Phyllomedusa* (Amphibia). *Journal of Herpetology* 28, 425–430.

Holloway, A.K., Cannatella, D.C., Gerhardt, H.C. & Hillis, D.M. (2006). Polyploids with different origins and ancestors form a single sexual polyploid species. *The American Naturalist* 167, 88–107.

Jackson, R.C. & Casey, J. (1982). Cytogenetic analyses of autopolyploids: models and methods for triploids to octoploids. *American Journal of Botany* 69, 487–501.

Jackson, R.C. & Hauber, D.P. (1982). Autotriploid and autotetraploid cytogenetic analyses: correction coefficients for proposed binomial models. *American Journal of Botany* 69, 644–646.

Jackson, R.C. & Hauber, D.P. (1994). Quantitative cytogenetic analyses of autoploid and alloploid taxa in the *Helianthus ciliaris* group (Compositae). *American Journal of Botany* 81, 1063–1069.

Jones, G.H. & Vincent, J.E. (1994). Meiosis in autopolyploid *Crepis capillaris*. II. Autotetraploids. *Genome* 37, 497–505.

King, M. (1990). *Animal Cytogenetics. Volume 4, Chordata 2, Amphibia*. Berlin: John.

Leggatt, R.A. & Iwama, G.K. (2003). Occurrence of polyploidy in the fishes. *Reviews in Fish Biology and*

Fisheries 13, 237–246.

Mable, B.K. (2003). Breaking down taxonomic barriers in polyploidy research. *Trends in Plant Science* 8, 582–590.

Mable, B.K. (2004). 'Why polyploidy is rarer in animals than in plants': myths and mechanisms. *Biological Journal of the Linnean Society* 82, 453–466.

Mable, B.K. & Roberts, J.D. (1997). Mitochondrial DNA evolution of tetraploids in the genus *Neobatrachus* (Anura: Myobatrachidae). *Copeia* 1997, 680–689.

Mahony, M.J. & Robinson, E.S. (1980). Polyploidy in the Australian leptodactylid frog genus *Neobatrachus*. *Chromosoma* 81, 199–212.

Matzke, M.A., Mittelsten Scheid, O. & Matzke, A.J.M. (1999). Rapid structural and epigenetic changes in polyploid and aneuploid genomes. *BioEssays* 21, 761–767.

Mendes Carneiro, S. (1975). Observations on the germ cell ultrastructure of male diploid and tetraploid *Odontophrynus americanus* (Amphibia: Anura). *Memorias del Instituto Butantan* 39, 135–148.

Muller, H.J. (1925). Why polyploidy is rarer in animals than in plants. *American Naturalist* 59, 346–353.

Orellana, J. & Santos, J.L. (1985). Pairing competition between identical and homologous chromosomes in autotetraploid rye. I. Submetacentric chromosomes. *Genetics* 111, 933–944.

Otto, S.P. & Whitton, J. (2000). Polyploid incidence and evolution. *Annual Review of Genetics* 34, 401–437.

Ptacek, M.B., Gerhardt, H.C. & Sage, R.D. (1994). Speciation by polyploidy in treefrogs: multiple origins of the tetraploid, *Hyla versicolor*. *Evolution* 48, 898–908.

Qu, L., Hancock, J.F. & Whallon, J.H. (1998). Evolution in an autopolyploid group displaying predominantly bivalent pairing at meiosis: genomic similarity of diploid *Vaccinium darrowi* and autotetraploid *V. corymbosum* (Ericaceae). *American Journal of Botany* 85, 698–703.

Rahn, I.M. & Martínez, A. (1983). Chromosome pairing in female and male diploid and polyploid anurans (Amphibia) from South America. *Canadian Journal of Genetics and Cytology* 25, 487–494.

Rosset, S.D., Baldo, D., Lanzone, C. & Basso, N.G. (2006). Review of the geographic distribution of diploid and tetraploid populations of the *Odontophrynus americanus* species complex (Anura: Leptodactylidae). *Journal of Herpetology* 40, 465–477.

Ruiz, I.R.G., Soma, M. & Beçak, W. (1981). Nucleolar organizer regions and constitutive heterochromatin in polyploid species of the genus *Odontophrynus* (Amphibia, Anura). *Cytogenetics and Cell Genetics* 29, 84–98.

Schmid, M. (1978). Chromosome banding in Amphibia II. Constitutive heterochromatin and nucleolus organizer region in Ranidae, Microhylidae and Racophoridae. *Chromosoma* 68, 131–148.

Schmid, M., Haaf, T. & Schempp, W. (1985). Chromosome banding in Amphibia IX. The polyploid karyotypes of *Odontophrynus americanus* and *Ceratophrys ornata* (Anura, Leptodactylidae). *Chromosoma* 91, 72–184.

Schmid, M., Steinlein, C. & Haaf, T. (2003). Chromosome banding in Amphibia XXVII. DNA replication banding patterns in three anuran species with greatly differing genome sizes. *Cytogenetic and Genome Research* 101, 54–61.

Sokal, R.R. & Rohlf, F.J. (1998). *Biometry*. San Francisco: Freeman.

Soltis, D.E. & Soltis, P.S. (1995). The dynamic nature of polyploid genomes. *Proceedings of the National Academy of Sciences USA* 92, 8089–8091.

Soltis, D.E. & Soltis, P.S. (1999). Polyploidy: recurrent formation and genome evolution. *Tree* 14, 348–352.

Stöck, M., Lamatsch, D.K., Steinlein, C., Epplen, J.T., Grosse, W-R., Hock, R., Klapperstück, T., Lampert, K.P., Scheer, U., Schmid, M. & Schartl, M. (2002). A bisexually reproducing all-triploid vertebrate. *Nature Genetics* 30, 325–328.

Sybenga, J. (1975). *Meiotic Configurations*. New York: Springer-Verlag.

Tymowska, J. (1991). Polyploidy and cytogenetic variation in frogs of the genus *Xenopus*. In *Amphibian Cytogenetics and Evolution*, 259–297. Green, D.M. & Sessions, S.K. (eds). California: Academic Press.

White, M.J.D. (1954). *Animal Cytology and Evolution*. Cambridge: Cambridge University Press.

Wolfe, K.H. (2001). Yesterday's polyploids and the mystery of diploidization. *Nature Reviews Genetics* 2, 333–341.

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