

Meiotic behavior of two polyploid species of genus *Pleurodema* (Anura: Leiuperidae) from central Argentina

NANCY E. SALAS¹, JULIÁN A. VALETTI², PABLO R. GRENAT^{1,3,*}, MANUEL A. OTERO¹, ADOLFO L. MARTINO¹

¹Ecología-Educación Ambiental, Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional N° 8 - km 601, (X5804BYA) Río Cuarto, Argentina. *Corresponding author. Email: pgrenat@exa.unrc.edu.ar

²Genética de Poblaciones, Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional N° 8 - km 601, (X5804BYA) Río Cuarto, Argentina

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Abstract. Polyploidy is an important evolutionary force but rare in vertebrates. However, in anurans, the genus *Pleurodema* has polyploid species, two of them tetraploid and one octoploid. The manner in which the chromosomes join in diakinesis can vary among species and, crucially, if they differ in their ploidy levels. In this work, we describe the meiotic configurations in two cryptic species from central Argentina, with different ploidy levels, *Pleurodema kriegi* (tetraploid) and *P. cordobae* (octoploid). A total of 306 diakineses from 19 individuals were analyzed. In meiosis, *P. kriegi* form 22 bivalents, whereas *P. cordobae* exhibits variation in meiotic figures. We discuss the possible allo- and autopolyploid origin of these species, and we consider that the autopolyploid origin of *P. cordobae* from *P. kriegi* might be the most feasible.

Keywords. *Pleurodema cordobae*, polyploidy, cytogenetics, diakinesis.

Polyploidy is an important evolutionary force in some plant groups, (White, 1973; Lacadena, 1996; Otto and Whitton, 2000), but this mechanism is rarer in vertebrates (Orr, 1990; Holloway et al., 2006). However, polyploidy in anuran amphibians has been documented in numerous families (Barrio and Rinaldi de Chieri, 1970; Bogart, 1980; Kawamura, 1984; Kuramoto, 1990; Beçak and Beçak, 1998; Otto and Whitton, 2000; Martino and Sinsch, 2002; Stöck et al., 2002; Rosset et al., 2006; Mable et al., 2011; Bogart and Bi, 2013).

The frog genus *Pleurodema* Tschudi, 1838 is distributed from Panama throughout South America to southern Chile and Argentina and currently is represented by 15 species, of which 10 have been recorded in Argentina (Valetti et al., 2009; Kolenc et al., 2009; Maciel and Nunes, 2010; Faivovich et al., 2012). This genus is exceptional in including species with three levels of ploidy.

According to cytogenetic studies conducted so far, *Pleurodema* includes three polyploid species, *P. kriegi* (Müller 1926) and *P. bibroni* Tschudi 1838 which are $2N = 4X = 44$, and *P. cordobae* Valetti, Salas and Martino 2009 which is $2N = 8X = 88$, while the remaining species are diploid ($2N = 2X = 22$) (Brum-Zorrilla and Sáez, 1968; Barrio and Rinaldi de Chieri, 1970; Veloso et al., 1972; Duellman and Veloso, 1977; Schmid et al., 1993; Lourenço et al., 2006; Valetti et al., 2009).

During meiosis, chromosome pairing and crossing over of homologous chromosomes occurs in prophase I, and is in diakinesis as can be seen by the more condensed chromosomes and the formation of chiasmata (Macgregor, 1993). The way in which chromosomes are joined forming chiasmata may vary between species, particularly if they are of different ploidy levels, resulting in different meiotic configurations (Salas and Martino, 2009).

In this paper, we analyze the meiotic figures of *Pleurodema kriegi* (tetraploid) and *Pleurodema cordobae* (octoploid).

Pleurodema kriegi and *P. cordobae* are two polyploid cryptic species endemic to the Sierra Grande of Cordoba, Argentina. Despite having different ploidy levels, they maintain a high degree of morphological and acoustical similarity (Valetti et al., 2009). The size of adult individuals in both species varies between 28 and 41 mm, *P. cordobae* is slightly larger than *P. kriegi* and, in both cases, females are larger than males (Valetti, 2012). These species breed from November to March (austral spring-summer) and are crepuscular and nocturnal (Valetti et al., 2009).

Samples of *P. kriegi* from two localities of Sierra de Achala (31°36'46" S, 64°52'29" W and 31°36'23" S, 64°52'6" W) and *P. cordobae* from two sites of Sierra de Comechingones (32°23'58" S, 64°55'35" W and 32°22'13" S, 64°55'55" W) were collected during spring and summer months between 2006 and 2010.

The Sierra de Córdoba constitutes the eastern group of the Sierras Pampeanas system embracing the Sierra Grande, Sierra Norte and Sierra Chica. Sierra Grande includes the Sierra de Achala to the north and the Sierra de Comechingones to the south (Miró, 1999; Ramos, 1999). This region is characterized by a humid temperate climate with snow in winter. The annual rainfall is 800–900 mm, distributed mainly in the spring-summer period (Capitanelli, 1979). The environment both in the Pampa de Achala and the Sierra de Comechingones is very similar. The water bodies in which the species were sampled were semi-permanent ponds in highland pastures.

After treatment in vivo with 0.3% colchicine, individuals were anesthetized and sacrificed by immersion in a 1% solution of MS-222 (Pukhta and Blazhek, 2004). Meiotic chromosomes, prepared from testis, were studied in 64 spermatocytes in diakinesis of four *P. kriegi* individuals and 242 spermatocytes in diakinesis from 15 *P. cordobae* individuals. All techniques, hypotonic treatment, fixation of cells and staining were performed according to Schmid, (1978a, b); Schmid et al., (1979) and Schmid et al., (1990). Chromosomal preparations, when dried and stained, were examined using a Zeiss Axiophot D-7082 fluorescence microscope with a Zeiss Axiocam HRC digital camera. Images were captured using the software AxioVision version 4.8. The diakineses were examined using the image analysis program Adobe® Photoshop™ CS2.

The analysis of spermatocytes in diakinesis revealed that all individuals of the tetraploid species *P. kriegi* formed 22 bivalents rings (Fig. 1A, 2A). In contrast, the analysis of diakinesis in individuals of *P. cordobae* revealed different meiotic configurations (Table 1). The

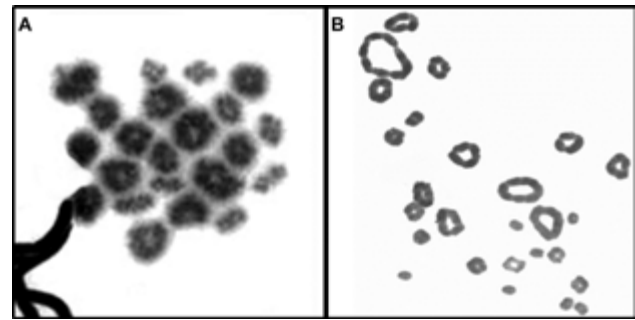


Fig. 1. Spermatocytes in diakinesis. (A) *Pleurodema kriegi*, La Posta, Pampa de Achala, ($2N = 4x = 44$). (B) *Pleurodema cordobae*, Los Tabaquillos, Sierra de Comechingones, ($2N = 8x = 88$).

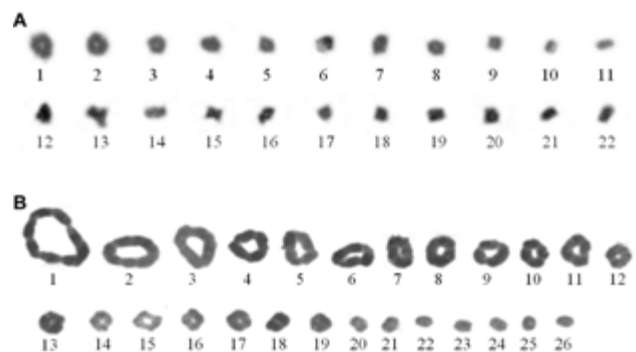


Fig. 2. Karyotype of the diakinesis of (A) *Pleurodema kriegi*, La Posta, Pampa de Achala, ($2N = 4x = 44$) and (B) *Pleurodema cordobae*, Los Tabaquillos, Sierra de Comechingones, ($2N = 8x = 88$).

Table 1. Patterns of meiotic figures in *Pleurodema cordobae*.

Meiotic configurations			Total observed diakineses
1 VIII	15 IV	10 II	165
1 VIII	14 IV	12 II	30
1 VIII	16 IV	8 II	25
15 IV	14 II		6
13 IV	18 II		6
1 VIII	13 IV	14 II	5
1 VIII	14 IV	12 II	5

most common configuration (68%) comprised an octavalent (VIII), 15 tetravalents (IV) and 10 bivalents (II) (Fig. 1B, 2b). However, other figures were observed. In 23% of the cases two types of configurations were observed: 1) an octavalent, 14 tetravalent and 12 bivalents; and 2) an octavalent, 16 tetravalents and eight bivalents. A small number (9%) of cells analyzed correspond to other types of configurations (Table 1).

Diakinetic analysis demonstrated that the chromosomes of tetraploid *P. kriegi* join forming bivalent instead of tetravalent figures, leading to 22 rings in all cases analyzed. Barrio and Rinaldi de Chieri (1970) interpreted these same results as reflecting an allopolyploid origin. Barrio (1977) suggested that the ancestral species of *P. kriegi* and *P. bibroni* (both tetraploid) would have originated by allopolyploidy from *P. thaul* and related species (possibly extinct) in semiarid western Argentina, and subsequently spread eastward from the plains to the Atlantic coast. Finally, in the Pleistocene, the ancestral species would have undergone cladogenesis, giving rise to the extant species *P. kriegi* in the central area of Argentina and *P. bibroni* in Uruguay.

In the analysis of *P. cordobae* diakinesis, chromosomes showed no consistent pattern. In contrast, we observed different numbers of rings in their cells, including multivalent meiotic configurations. Multivalent configurations have been described in several polyploid anuran species (Beçak et al., 1966, 1967, 1970; Schmid et al., 1985; Salas and Martino, 2009). Although multivalent formations can occur in an allopolyploid if the hybridizing species are closely related (Chenuil et al., 1999), the multivalent formations observed in *P. cordobae* could be evidence of autopolyploid origin. Moreover, recently Faivovich et al., (2012) performed a phylogenetic analysis of the genus *Pleurodema* based on sequences of nuclear and mitochondrial genes. These authors obtained a clade comprising the three polyploid species, where *P. cordobae* is the sister of *P. kriegi* and these two species together form the sister taxon of *P. bibroni*. As the authors demonstrate, these results also would be consistent with an autopolyploid origin of *P. cordobae* from *P. kriegi*, although they make clear that cytogenetic studies and a population-level approach will be required to confirm this. Therefore, under the hypothesis of a recent autopolyploid origin of *P. cordobae* from *P. kriegi*, both geographically close, it could be expected that the chromosomes are paired forming tetrads, in diakinesis. This is because in the potential ancestral species the chromosomes are paired forming dyads. However, as noted above, the behavior of the chromosomes was not as expected. Bogart and Bi (2013) conclude that polyploid species generally have higher levels of genetic diversity than related diploid species and that these polyploids accumulate genes from such diploid associations by having additional linkage groups. Therefore, meiotic aberrations are necessary for the initial evolution of polyploids and may persist to maintain the genomic integrity (Bogart and Bi, 2013; Stenberg and Saura, 2013). Consequently, the origin of *P. cordobae* by polyploidy could be relatively recent and the meiotic behavior to be responding to the same fact. Although in *P. cordobae* we did not observe a consistent pattern, the presence of an octavalent ring in all diakinesis tested is nota-

ble. However, in accordance with expected pattern and the studies realized to date, we cannot interpret such meiotic behavior. Cytogenetic studies by GISH technique in cells of *P. cordobae* hybridized with probe of total genomic DNA of *P. kriegi*, would determine the number of chromosome complements shared by the two species. This method would enable evaluate the hypothesis of an autopolyploid origin of *P. cordobae* from *P. kriegi*.

The polyploid individuals produce viable gametes if these are balanced in chromosome number; otherwise the gametes would be infertile. Macgregor (1993) stated that this problem could be solved by mechanisms to prevent unequal multivalent formation, or by strengthening the effective meiosis diploidization yielding only bivalents. The results obtained from our analysis of meiotic configurations of *P. cordobae* may indicate that the gametes formed in this species have low viability. However, populations of *P. cordobae* are quite numerous and biological data on the species indicate that these populations are reproducing without major problems (Valetti et al., 2011). Future studies analyzing chromosome behavior in diakinesis in greater detail and evaluating fertility in *P. cordobae* will be critical to the understanding of this particular chromosome pairing mode at the stage prior to the generation of gametes. Moreover, the octoploid condition of *P. cordobae* and the features shown in this study make this species an interesting model for genetic research.

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REFERENCES

- Barrio, A. (1977): Aportes para la elucidación del “status” taxonómico de *Pleurodema bibroni* Tschudi y *Pleurodema kriegi* (Müller) (Amphibia, Anura, Leptodactylidae). *Physis* **37**: 311-331.
- Barrio, A., Rinaldi de Chieri, P. (1970): Estudios citogenéticos sobre el género *Pleurodema* y sus consecuencias evolutivas (Amphibia, Anura, Leptodactylidae). *Physis* **30**: 309-319.
- 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. (1967): Further studies on polyploid amphibians (Ceratophryidae). I. Mitotic and meiotic aspects. *Chromosoma* **22**: 192-201.
- Beçak, M.L., Denaro, L., Beçak, W. (1970): Polyploidy and mechanisms of karyotypic diversification in Amphibia. *Cytogenetics* **9**: 225-238.
- Beçak, M.L., Beçak, W. (1998): Evolution by polyploidy in Amphibia: New insights. *Cytogenet. Cell Genet.* **80**: 28-33.
- Bogart, J.P. (1980): Evolutionary significance of polyploidy in amphibians and reptiles. *Polyploidy: biological relevance*. Plenum Press, New York.
- Bogart, J.P., Bi. K. (2013): Genetic and genomic interactions of animals with different ploidy levels. *Cytogenet. Genome Res.* **140**: 117-136.
- Brum-Zorrilla, N., Sáez, F.A. (1968): Chromosomes of Leptodactylidae (Amphibia Anura). *Experientia* **24**: 969.
- Capitanelli, R.G. (1979): Clima. In: Geografía física de la provincia de Córdoba, pp. 45-138. Vázquez, J.B., Miatello R.A., Roqué, M.E., Eds., Editorial Boldt, Buenos Aires.
- Cei, J.M. (1972): Segregación corológica y procesos de especiación por aislamiento en anfibios de la Pampa de Achala, Córdoba. *Acta Zool. Lilloana* **29**: 233-246.
- Chenuil, A., Galtier, N., Berrebi, P. (1999): A test of the hypothesis of an autopolyploid vs. allopolyploid origin for a tetraploid lineage: application to the genus *Barbus* (Cyprinidae). *Heredity* **82**: 373-380.
- Duellman, W.E., Veloso, A. (1977): Phylogeny of *Pleurodema* (Anura: Leptodactylidae): A biogeographic model. *Occas. Pap. Mus. Nat. Hist. Univ. Kans.* **64**: 1-46.
- Faivovich, J., Ferraro, D.P., Basso, N. G., Haddad, C.F.D., Rodrigues, M.T., Wheeler, W.C., Lavilla, E.O. (2012): A phylogenetic analysis of *Pleurodema* (Anura: Leptodactylidae: Leiuperinae) based on mitochondrial and nuclear gene sequences, with comments on the evolution of anuran foam nests. *Cladistics* **28**: 460-482.
- 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. *Am. Nat.* **167**: 88-101.
- Kawamura, T. (1984): Polyploidy in amphibians. *Zool. Sci.* **1**: 1-5.
- Kolenc, F., Borteiro, C., Baldo, D., Ferraro, D.P., Prigioni, C. (2009): The tadpoles of *Pleurodema bibroni* Tschudi and *Pleurodema kriegi* (Müller), with notes on their advertisement calls, natural history and conservation status (Amphibia, Anura, Leiuperidae). *Zootaxa* **1969**: 1-35.
- Kuramoto, M. (1990): A list of chromosome numbers of anuran amphibians. *Bull. Fukuoka Univ. Edac.* **39**: 83-127.
- Lacadena, J.R. (1996): *Citogenética*. Editorial Complutense S.A., Madrid.
- Lourenço, L.B., Nascimento, J.A., Andrade, G.V., Rosa-Feres, D.C., Recco-Pimentel, S.M. (2006): Chromosomal analysis of the leptodactylids *Pleurodema diplolistris* and *Physalaemus nattereri* (Amphibia, Anura). *Amphibia-Reptilia* **27**: 481-489.
- Mable, B.K., Alexandrou, M.A., Taylor, M.I. (2011): Genome duplication in amphibians and fish: an extended synthesis. *J. Zool.* **284**: 151-182.
- Macgregor, H.C. (1993): *An Introduction to Animal Cytogenetics*. Chapman and Hall, London.
- Maciel, D.B., Nunes, I. (2010): A new species of four-eyed frog genus *Pleurodema* Tschudi, 1838 (Anura: Leiuperidae) from the rock meadows of Espinhaço range, Brazil. *Zootaxa* **2640**: 53-61.
- Martino, A.L., Sinsch, U. (2002): Speciation by polyploidy in *Odontophrynus americanus*. *J. Zool.* **257**: 67-81.
- Miró, R.C. (1999): El basamento Precámbrico-Paleozoico inferior de las Sierras Pampeanas, Famatina, Cordillera Oriental y Puna. *Geología Argentina. Anales*, **29**: 133-167.
- Müller, L. (1926): Neue Reptilien und Batrachier der Zoologischen Sammlung des bayrischen Staates. *Zool. Anz.* **65**: 193-200.
- Orr, H.A. (1990): Why polyploidy is rarer in animals than in plants. Revisited. *Am. Nat.* **136**: 759-770.
- Otto, S.P., Whitton, J. (2000): Polyploid incidence and evolution. *Annu. Rev. Genet.* **34**: 401-437.
- Pukhta, V., Blazhek, I.Y. (2004): Use of the ethyl ether of metaaminobenzoic acid (MS-222) for general anesthesia of cold-blooded animals. *Bull. Exp. Biol. Med.* **53**: 356-358.
- Ramos, V.A. (1999): Rasgos estructurales del territorio Argentino. I. Evolución tectónica de la Argentina. *Geología Argentina. Anales*. **29**: 715-786.
- 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). *J. Herpetol.* **40**: 465-477.
- Salas, N.E., Martino, A.L. (2009): Patrones de configuraciones meióticas en poblaciones de *Odontophrynus cordobae* y *O. americanus* (Anura: Cycloramphidae), en el área central de Argentina. *J. Basic Appl. Genet.* **19**: 27-32.
- Schmid, M. (1978a): Chromosome banding in amphibia. I. Constitutive heterochromatin and nucleolus organizer regions in *Bufo* and *Hyla*. *Chromosoma* **66**: 361-388.

- Schmid, M. (1978b): Chromosome banding in Amphibia II. Constitutive heterochromatin and nucleolus organizer regions in Ranidae, Microhylidae and Rhacophoridae. *Chromosoma* **68**: 131-148.
- Schmid, M., Olert, J., Klett, C. (1979): Chromosome banding in Amphibia III. Sex chromosomes in *Triturus*. *Chromosoma* **71**: 29-55.
- 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**: 172-184.
- Schmid, M., Steinlein, C., Nanda, Y., Epplen, J. (1990): Chromosome banding in Amphibia. In: Cytogenetics of Amphibians and Reptiles, pp. 21-54. Olmo E., Ed., Advances in Life Science, Birkhauser Verlag, Basel.
- Schmid, M., Steinlein, C., Feichtinger, W., Poot, M. (1993): Chromosome banding in Amphibia. XVIII. Karyotype evolution and genomic size variation in *Pleurodema* (Anura, Leptodactylidae). *Cytogenet. Cell Genet.* **62**: 42-48.
- Stenberg, P., Saura, A. (2013). Meiosis and its deviations in polyploid animals. *Cytogenet. Genome Res.* **140**: 185-203.
- Stöck, M., Lamatsch, D.K., Steinlein, C., Epplen, J.P., Grosse, W.R., R. Hock, R., Klapperstück, T., Lampert, K.P., Scheer, U., Schmid, M., Schartl, M. (2002): A bisexually reproducing all-triploid vertebrate. *Nature Genet.* **30**: 325-328.
- Valetti, J.A. (2012): Biodiversidad del género *Pleurodema* en el área central de Argentina y análisis comparativo de los diferentes *taxa* mediante el uso de caracteres morfológicos, celulares y etológicos. Tesis Doctoral. Universidad Nacional de Río Cuarto, Río Cuarto, Argentina.
- Valetti, J.A., Salas, N.E., Martino, A.L. (2009): A new polyploid species of *Pleurodema* (Anura: Leiuperidae) from Sierra de Comechingones, Córdoba, Argentina and redescription of *Pleurodema kriegi* (Müller, 1926). *Zootaxa.* **2073**: 1-21.
- Valetti, J.A., Otero, M.A., Grenat, P.R., Martino, A.L. (2011): Clutch size. *Pleurodema cordobae* (Octoploid *Pleurodema*). *Herpetol. Rev.* **42**: 413.
- Veloso, A.M., Diaz, N.P., Galleguillos, R.G. (1972): Revisión cariosistémática de las especies del género *Pleurodema* en Chile (Anura: Leptodactylidae). *An. Mus. Hist. Nat. Valparaiso.* **6**: 49-55.
- White, M.J.D. (1973): Animal cytology and evolution. Cambridge University Press, London.

