

## Short Notes

# Natural interspecific hybridization in *Odontophrynus* (Anura: Cycloramphidae)

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**Abstract.** The frog genus *Odontophrynus* is a composite of diploid and tetraploid populations and species that are widely distributed in South America. Some of the several genetic studies on this group report the production of artificial hybrids but only a single case of natural hybridization has been documented, in southern Brazil. In this study we report the finding of an interspecific natural hybrid specimen in central Argentina. We present morphological and cytogenetical evidence that the diploid taxa *Odontophrynus cordobae* and *O. occidentalis* are the parental species. The hybrid genome exhibited problematic pairing and segregation of homeologue chromosomes during meiosis, and the production of non-reduced gametes.

**Keywords:** cytogenetic, hybridization, meiosis, morphology, *Odontophrynus*, polyploidy.

The Neotropical genus *Odontophrynus* currently comprises 11 nominal species distributed in southern and eastern South America (Frost, 2008), which are arranged in four phenetic groups: *americanus*, *cultripes*, *moratoi*, and *occidentalis* (Savage and Cei, 1965; Cei, 1987; Caramaschi, 1996). *Odontophrynus americanus* was the first reported case of a bisexual polyploid vertebrate (Beçak, Beçak and Rabello,

1966) and since then, a vast number of genetic studies were done on this genus (for review see Beçak and Beçak, 1998 and Rosset et al., 2006). Some of these studies reported the production of artificial hybrids, but only a single case of natural interspecific hybridization, originated by mating between tetraploid *Odontophrynus americanus* and diploid *O. cultripes*, was reported in southern Minas Gerais, Brazil (Ruiz, Bonaldo and Beçak, 1980, and references herein). In the present study we provide morphological and cytogenetical evidence of a new case of natural hybridization in *Odontophrynus* between two diploid taxa, *O. cordobae* (*americanus* group) and *O. occidentalis* (*occidentalis* group), with some remarks about chromosome behaviour during the meiotic division.

During field surveys in the Sierras Pampeanas orographic system, one adult male of uncertain taxonomic status was collected. It was found calling in a low flow temporary stream in syntopy with specimens of *O. cordobae* and *O. occidentalis*, at Villa Flor Serrana, Córdoba province, Argentina (31°22'53.42"S; 64°35'38.64"W; 875 m.a.s.l.), on September 12, 2007. This specimen, a male of *O. cordobae* and a male of *O. occidentalis* were collected to study their morphology and karyology. Additional individuals of *Odontophrynus cordobae* and *O. occidentalis* from Villa Flor Serrana and other localities from Argentina were studied mor-

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phologically and karyotyped (Appendix 1). Specimens were killed with 5% lidocaine, fixed in 10% formalin, stored in ethanol 70°, and were deposited in Diego Baldo personal collection (MLP DB), housed at Museo de La Plata, Argentina.

Examination of exosomatic characters was done through a stereoscopic microscope NIKON SMZ 445. Preparations of mitotic and meiotic chromosomes were obtained from bone marrow and testicle cellular suspensions respectively, following Schmid (1978). Preparations were dropped over slides and stained with a 10% Giemsa solution with phosphate buffer. The location of Nucleolus Organizer Regions (NORs) in metaphasic chromosomes was detected by silver staining following the technique described by Howell and Black (1980). Mitotic and meiotic images were observed under an OLYMPUS BX50F-3 photomicroscope and photomicrographs were taken with a SONY ExwaveHAD videocamera. The biometric analysis of chromosomes was done using MicroMeasure v3.3 software (Reeves and Tear, 2000). Karyotypes were arranged and chromosomes classified after Levan, Fredga and Sandberg (1964). The terms  $x$  (basic chromosome number),  $n$  (gametic chromosome number),  $2n$  (somatic chromosome number), and FN (fundamental number) were used as suggested by White (1954).

The analysis of the external morphological characters in the exemplar MLP DB 6508 (fig. 1A), revealed the presence of the following features that are shared with *Odontophrynus cordobae* (fig. 1B): (1) elongated postorbital and parotoid glands; (2) round temporal glands; (3) coloration pattern with dark brown round spots, bordered by black, that are nearly symmetrical on the dorsum, over a light brown background colour; (4) dark brown spots on the maxilla and the dorsum of the head (Martino and Sinsch, 2002; Rosset, 2008). In addition, the following characters of this individual are similar to those present in *Odontophrynus occidentalis* (fig. 1C): (1) a pair of relatively large

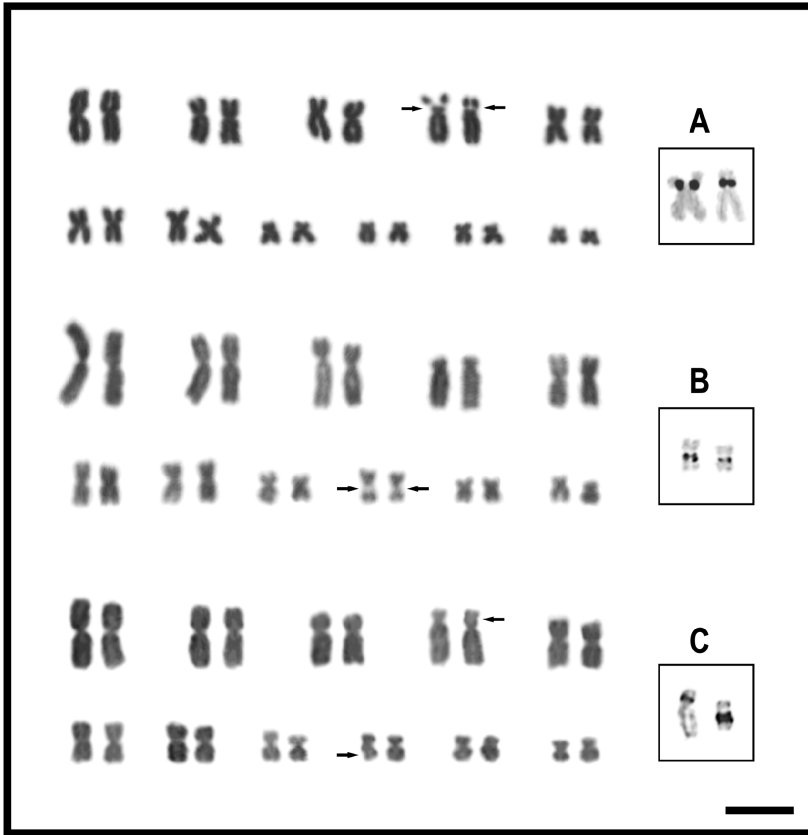
glands on the upper eyelid; (2) tibial glands; (3) a large number of rounded glands on both sides of the body; (4) large keratinous nuptial spines on the dorsum (Savage and Cei, 1965; Cei, 1987; Rosset et al., 2007).

The studied karyotypes of *Odontophrynus cordobae* and *O. occidentalis* were composed by  $2n = 2x = 22$  bichromosomes (FN = 44), consisting of four large pairs, three medium pairs, and four small pairs of elements. In *O. cordobae*, the pairs 1-2, 5-7, and 11 were metacentric, and pairs 3-4 and 8-10 were submetacentric, with Secondary Constrictions (SC) in the interstitial region on the short arm of pair 4 and, less commonly with an additional SC in the interstitial region of the long arm of pair 9 (fig. 2A). In *O. occidentalis*, the pairs 1-2, 5-9, and 11 were metacentric, pairs 3 and 10 submetacentric, and pair 4 subtelocentric, with SC in the interstitial region of the long arm of pair 9 (fig. 2B). The silver staining revealed differences in the position of the NORs between species, that were coincident with the position of the SC of pair 4 in *O. cordobae* and of pair 9 in *O. occidentalis*. All these karyotypic features are consistent with previous works (Beçak and Beçak, 1974; Ruiz, Soma and Beçak, 1981; Ruiz, Cei and Beçak, 1982; Rosset et al., 2006).

The karyotype of specimen MLP DB 6508 presented chromosome heteromorphism in pairs 4, 8, and 9 due to different chromosome morphology and position of the SC and NORs (fig. 2C). This heteromorphism between the two haploid chromosomal sets is in full agreement



**Figure 1.** Lateral views of hybrid specimen (MLP DB 6508) (A) and parental species: *Odontophrynus cordobae* (MLP DB 6507) (B), and *O. occidentalis* (MLP DB 6506) (C).

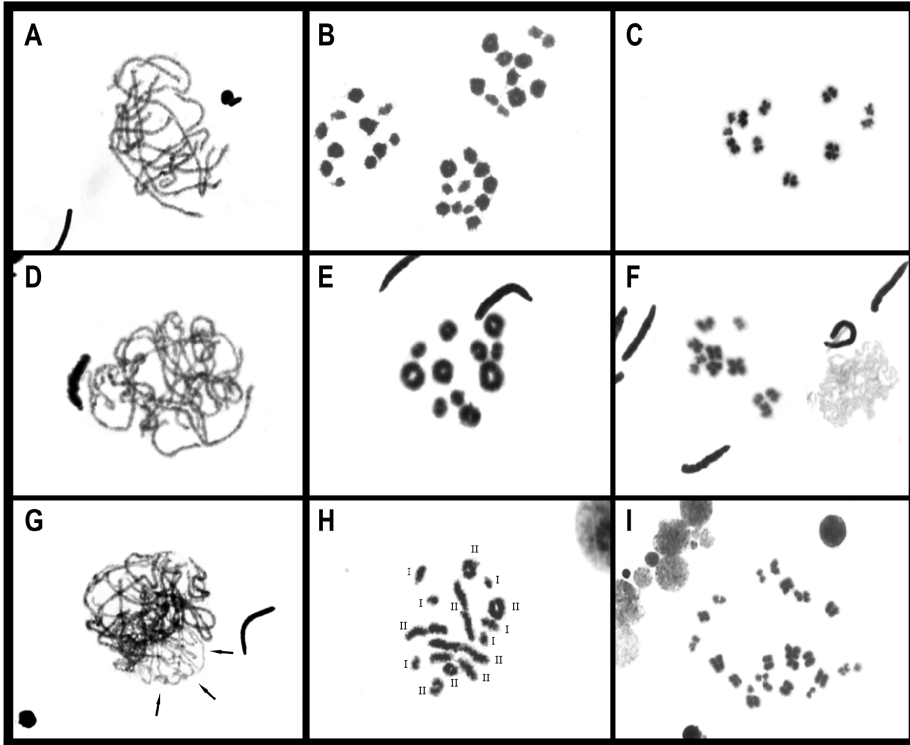


**Figure 2.** Giemsa stained karyotypes and NOR-bearing chromosome pairs (box) of (A) *Odontophrynus cordobae*; (B) *O. occidentalis*; and (C) hybrid specimen MLP DB 6508. Note the heteromorphism in the location in NORs in C. The arrows indicate the secondary constriction. Bar = 10  $\mu$ m.

with the differences observed between the karyotypes of *Odontophrynus cordobae* and *O. occidentalis*, and is conclusively supporting the hypothesis of hybrid origin.

The meiosis of *O. cordobae* and *O. occidentalis* displayed a complete chromosome pairing in zygotene-pachytene (fig. 3A, D), 11 bivalents in ring configuration with terminal chiasmata were observed during diakinesis (fig. 3B, E). On the other hand, some chromosomal regions remained unpaired during the late zygotene-pachytene in the hybrid specimen (fig. 3G). Moreover, in diakinesis a relatively high frequency of univalents (5.20/cell) as well as a decrease in the number of chiasmata were observed in this specimen (12.12/cell) (fig. 3H). In studied specimens of *O. cordobae* and *O. occidentalis* (6 and 13, respectively) we observed

19.47 chiasmata per cell, all of them with the same number of chromosome arms and potentially similar number of chiasmata. There were no statistical differences in chiasmata distribution (proximal-interstitial-distal),  $\chi^2 = 1.55$ ,  $df = 2$ ,  $P = 0.46$  NS, between *O. cordobae* and *O. occidentalis*, and between both species pooled against MLP DB 6508,  $\chi^2 = 0.29$ ,  $df = 2$ ,  $P = 0.87$  NS. The anomalies observed in the hybrid specimen correspond to evident pairing problems of homeologous chromosomes during early pachytene stage, which have lead to the production of a high number of non-reduced gametes (58.93%) (fig. 3I), as was previously observed in artificial triploid hybrids *Odontophrynus cultripes*  $\times$  *O. americanus* (45.83%) (Beçak and Beçak, 1970). The formation of haploid, diploid and triploid gametes



**Figure 3.** Meiotic stages of *Odontophrynus cordobae* (A-C); *O. occidentalis* (D-F), and hybrid specimen (G-I): Late zygotene-pachytene (A, D and G), diakinesis (B, E and H), and metaphase II (C, F and I). Note the non-pairing regions of chromosomes in the hybrid exemplar in G (arrows) and the presence of univalents (I) and bivalents (II) in H. In I note the presence of a number non-reduced of chromosomes.

by artificial triploid hybrids led to propose that these eventual individuals could be an intermediary step to achieve tetraploidy (Beçak and Beçak, 1970; Beçak and Kobashi, 2004). Non-reduced gametes can be produced by distortions of cytokinesis and the formation of restitution nuclei, because of pairing failure and segregation lagging of chromosomes from the different parental species (Islam and Shepherd, 1980).

Our results provide the first documented case of a natural hybrid between *Odontophrynus cordobae* and *O. occidentalis*, which shares exosomatic and chromosomal characters with both species. Distortions in chromosomal behaviour during meiosis reflect differences between the homeologous chromosomes from each parental species, by-product of chromosomal rearrangements during the karyotypic evolution of each one. Depending on the grade of divergence of

parental genomes, these alterations in gamete formation would act as a postzygotic barrier, contributing to the isolation between species. Alternatively, the production of non-reduced gametes can be a first step towards the formation of an allopolyploid species if the zygotes produced by fecundation of non-reduced gametes can restore chromosomal pairing and segregation (Rieseberg, 2001). Additional and more detailed studies about reproductive aspects of hybrids would give new insights on the frequency and relevance of hybridization in nature and the effectiveness of reproductive isolation mechanisms to avoid it.

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## Appendix 1

Specimens studied morphologically (all) and cytogenetically (with asterisks).

*Odontophrynus cordobae*. – ARGENTINA: CÓRDOBA: Santa Catalina: MLP DB 2606-2609 (\*), 2851, 2907-2908 (\*); Villa Flor Serrana: MLP DB 4670-4671 (\*), MLP DB 6507 (\*).

*Odontophrynus occidentalis*. – ARGENTINA: CÓRDOBA: Quebrada del Cóndor, Rio Ceballos: MLP DB 2615 (\*), 2616; Cuesta Blanca: MLP DB 4672 (\*), 4673-4674, 4830; Villa Flor Serrana: MLP DB 6506 (\*); MENDOZA: El Nihuil, Rio Atuel: MLP DB 3268, 3275, 3282, 3312-3314 (\*), 3358, 3371, 3429; Valle Grande, Rincón del Atuel: MLP DB 3266-3268, 3275, 3277-3278, 3319-3321 (\*), 3352, 3357 (\*), 3388 (\*), 3389-3390, 3393, 3400-3401 (\*); SAN LUIS: Inti Huasi: MLP DB 3267; 3380 (\*), 3391, 3392 (\*), 3428, 3430; NEUQUÉN: Villa El Chocón: MLP DB 3729, 3730 (\*), 3731.

*Odontophrynus cordobae*  $\times$  *Odontophrynus occidentalis*. – ARGENTINA: CÓRDOBA: Villa Flor Serrana: MLP DB 6508 (\*).