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MITOTIC AND MEIOTIC CHROMOSOMES OF *ARTEMIA* (BRANCHIOPODA) FROM POPULATIONS OF LA PAMPA PROVINCE, ARGENTINA

S. Rodríguez Gil, A. G. Papeschi, and R. G. Cohen

ABSTRACT

Three populations of *Artemia* from La Pampa Province, Argentina, were cytogenetically analyzed: Salinas Grandes de Hidalgo, Laguna Callaqueo, and Laguna Colorada Chica. Both mitotic and male meiotic chromosomes were studied. All 3 populations share the same diploid (2n = 44) and/or haploid chromosome number (n = 22), and hence were determined as *Artemia persimilis* Piccinelli and Prosdocimi. Although bivalents decrease gradually in size, differences among the larger and the smaller ones were apparent. So far, no cytogenetic differences have been detected among the 3 populations. Chromosomes with metacentric, submetacentric, and telocentric morphology were detected in mitotic prometaphase cells, suggesting that chromosomes of *Artemia* may be monocentric.

The anostracan genus *Artemia* is taxonomically regarded as a group of several reproductively isolated sibling species, distributed worldwide, except for the Antarctic continent. In the New World, the genus is represented by three endemic bisexual species: *A. franciscana* Kellogg, 1906, *A. persimilis* Piccinelli and Prosdocimi, 1968, and *A. monica* Verrill, 1869.

Artemia franciscana is the dominant species in North and South America, as well as in the Caribbean (Vanhaecke *et al.*, 1987). Its broad distribution may be attributed to the deliberate introduction by man for commercial purposes and/or to ordinary natural dispersal by wind or by zoochory through waterfowl, such as the flamingo *Phoenicopterus chilensis* Molina (see Lenz and Browne, 1991). This anostracan is widely distributed in salt ponds in many South American countries through the extensive migrations of waterfowl, which passively seed brine-shrimp cysts. *Artemia monica* and *A. persimilis* are both restricted to unique sites: the first one to Mono Lake (California, U.S.A.) and the second one to various saline water bodies of Argentina (Vanhaecke *et al.*, 1987; Browne and Bowen, 1991; Amat *et al.*, 1994; Cohen, 1995). The above mentioned dispersal strategies, together with the strong presumption that *A. franciscana* is undergoing a process of incipient speciation (Beardmore *et al.*, 1996), denoted by a high degree of interpopulation diversity, make the study of species distribution patterns and identification of *Artemia* a remarkably difficult subject. The use of electrophoretic techniques has revealed the close phylogenetic relationship existing between a population of *Artemia* from Salar de Atacama, northern Chile (23°30′S, 68°10′W) and a population of *A. franciscana* from San Francisco Bay, U.S.A. (Gajardo and Beardmore, 1993). Colihueque and Gajardo (1996) recently found that in respect to chromosome and chromocenter numbers, the population from Salar de Atacama is closely related to *A. persimilis* from Buenos Aires Province, Argentina. Further confirmation of the similarities and divergences among these taxa is required.

Despite the abundance and diversity of saline ecosystems in Argentina, a screening of the genus *Artemia* is still lacking. Thus, the distribution of *A. persimilis* and whether *A. franciscana* is present in this country are still unknown topics.

Cytogenetic studies on *Artemia* have been hindered, due to its high chromosome number, small chromosome size, low mitotic index of nauplii, and frequent nonspecific chromosome associations (Barigozzi, 1974; Abatzopoulos *et al.*, 1986). These factors turn the morphological characterization of chromosomes of *Artemia* into a very difficult task. It has even been suggested that they could be holokinetic (Stefani, 1963a, b; Stefani and Cadeddu, 1967; Barigozzi, 1974). On the other hand, meiotic studies on the genus have been performed only on females (Halfer-Cervini *et al.*, 1968; Barigozzi, 1974).

Both *A. franciscana* and *A. persimilis* are diploid bisexual species, differing in their diploid chromosome number (2n = 42 and 2n = 44, respectively), and other cytogenetic

Table 1. Distribution of diploid chromosome numbers in populations of *Artemia*.

Locality	Stage	Chromosome number															
		<	38	39	40	41	42	43	**44**	45	46	47	48	49	50	>	Total cells
Hidalgo	Nauplii	6	3	3	0	0	1	0	**6**	0	2	1	3	0	0	0	25
	Adults	6	1	2	1	0	2	0	**4**	2	0	2	1	1	0	1	23
	Total	12	4	5	1	0	3	0	**10**	2	2	3	4	1	0	1	48
Callaqueo	Nauplii	0	0	0	0	0	0	0	**5**	0	0	0	0	0	0	0	5
	Adults	2	1	0	0	0	0	0	**2**	0	1	1	0	0	0	0	7
	Total	2	1	0	0	0	0	0	**7**	0	1	1	0	0	0	0	12

traits, such as average chromosome length, number of chromocenters in interphase nuclei, repetitive DNA content, etc. (Abreu-Grobois, 1987; Badaracco *et al.*, 1987). The present contribution aims at analyzing and comparing mitotic and male meiotic chromosomes of *Artemia* from three populations of La Pampa Province, Argentina, in order to describe their cytogenetic characteristics and determine their specific identity.

Materials and Methods

Samples of cysts of brine shrimp were collected from 3 populations of La Pampa Province, Argentina: Salinas Grandes de Hidalgo (37°13′S, 63°26′W), Laguna Callaqueo (38°34′S, 63°32′W), and Laguna Colorada Chica (38°23′S, 63°36′W).

Following the technique described by Colihueque and Gajardo (1996), mitotic cells were obtained from 12–24-h-old nauplii, reared at 25°C. These were treated with colchicine (0.1%) in sea water for 45 min and then transferred to a hypotonic treatment in lukewarm distilled water (38°C) for 90 min. The nauplii were then placed on a slide with a drop of lacto-propionic orcein. Three or four nauplii per slide were squashed under the coverslip.

Adult males were anaesthetized by cooling them, and then their testes were dissected out. Meiotic slides were prepared by placing a piece of gonad directly on a drop of lacto-propionic orcein, and by squashing the material, after slight dilaceration, under the coverslip.

The total number of adult males processed was: 48 from Hidalgo, 38 from Callaqueo, and 22 from Colorada Chica. However, only 11, 5, and 4 specimens, respectively, showed mitotic and/or meiotic cells suitable for chromosome analysis.

Results

The diploid chromosome number was determined, both in somatic cells and in spermatogonial cells, of specimens from Callaqueo and Hidalgo (Figs. 1A–D, 2A) (Table 1). Reliable mitotic cells were not observed in individuals from Colorada Chica. In the first two populations, the normal chromosome number was 2n = 44. Cells with more or fewer chromosomes were considered as technical artifacts (Table 1).

In metaphase, chromosomes were condensed and closely associated with each other, making their proper analysis impossible. However, in some cells in prometaphase the morphology of certain chromosomes appeared to be metacentric, submetacentric, and telocentric (Fig. 1C, E, F).

During male meiosis, cells in leptotene-zygotene and pachytene were observed (Fig. 3A). In diplotene, bivalents were faintly stained and nonspecific associations were frequent. In spite of the fact that bivalent morphology was very difficult to ascertain, 22 bivalents were clearly observed in diakinesis and prometaphase I (Figs. 2B, D, 3B, C). Although bivalents decrease gradually in size, differences among the larger and the smaller ones were apparent (Fig. 3E). In prometaphase II, cells with 22 chromosomes were readily seen (Figs. 2C, E, 3D). Table 2 summarizes the distribution of haploid chromosome numbers in cells in prometaphase I and prometaphase II. Bivalents in metaphase I and chromosomes in metaphase II were too packed to be accurately identified. No cell in anaphase I or II was observed. All slides presented a high number of spermatids.

Discussion

The three populations herein analyzed (Salinas Grandes de Hidalgo, Laguna Callaqueo, and Laguna Colorada Chica) show the diploid (2n = 44) and/or haploid (n = 22) chromosome number characteristic of *A. persimilis*. Our results are in agreement with the conclusions of a previous morphological analysis carried out by Amat *et al.* (1994) on the same populations.

Many authors have referred to the technical difficulties they confronted in order to obtain good metaphase plates from nauplii of *Artemia* (see Barigozzi, 1974; Amat, 1982; Abatzopoulos *et al.*, 1986). They mentioned

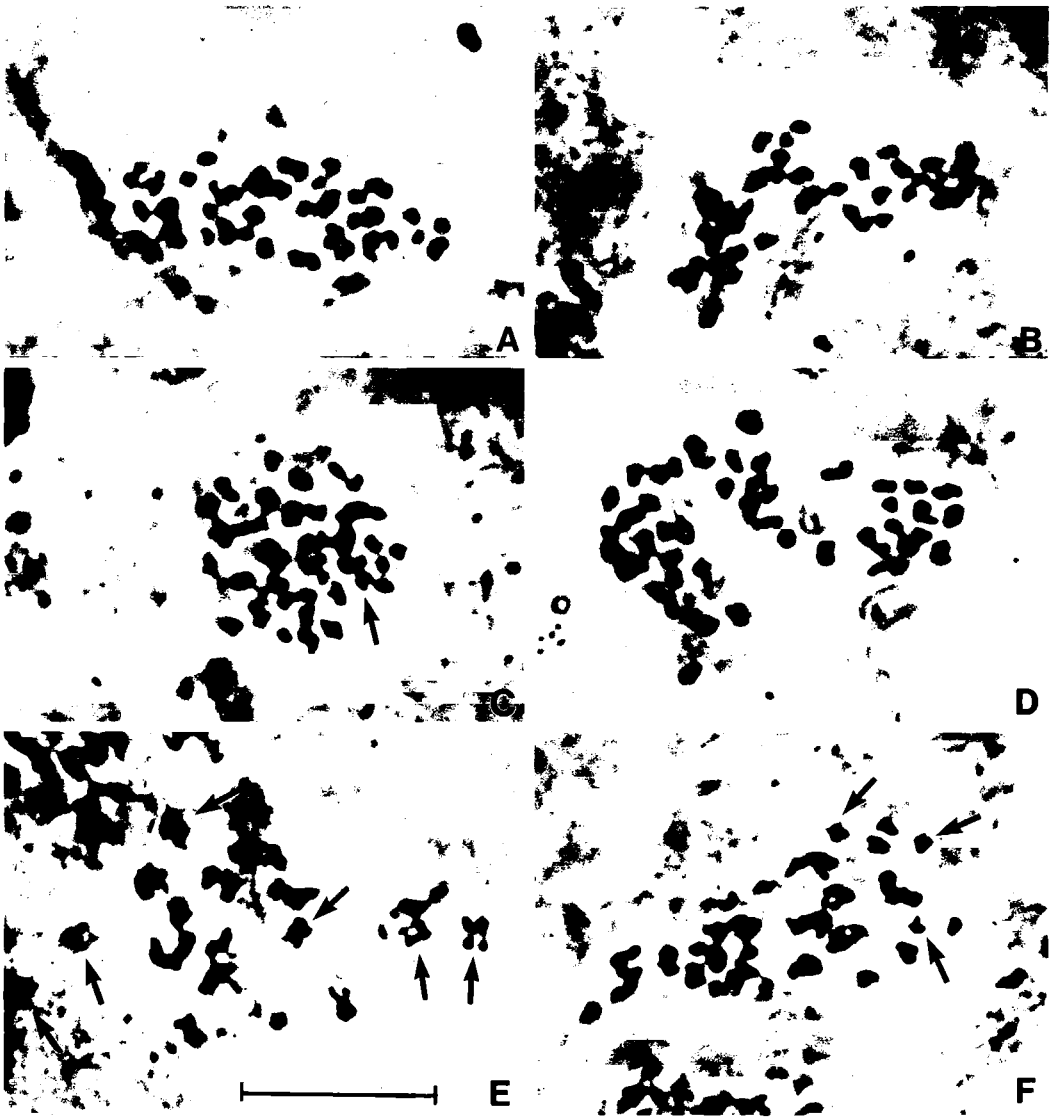

Fig. 1. Mitotic cells in nauplii of *Artemia* from Callaqueo (A–C) and Salinas Grandes de Hidalgo (D–F). In C, E, F the arrows indicate metacentric, submetacentric, and telocentric chromosomes. Bars in all figures represent 10 μm.

high chromosome number, small chromosome size, and frequent nonspecific associations among chromosomes as the main obstacles. In *A. persimilis,* technical difficulties are even greater than in *A. franciscana,* since its diploid chromosome number is higher (2n = 44 instead of 42) and its average chromosome length significantly shorter (Baratelli and Barigozzi, 1990). Chromosome size differences are also evident when comparing the

Table 2. Distribution of haploid chromosome numbers in populations of *Artemia.*

	Chromosome number									Total number	
Locality	<	19	20	21	**22**	23	24	25	>	cells	individuals
Hidalgo	1	1	3	1	**15**	0	0	0	2	23	**4**
Callaqueo	5	1	2	2	**14**	4	0	0	2	30	3
Colorada Chica	0	0	0	2	**16**	0	1	0	1	20	**4**
Total										73	

Fig. 2. *Artemia* from Callaqueo (A–C) and Salinas Grandes de Hidalgo (D–E). (A) Spermatogonial prometaphases with 44 chromosomes. (B–E) Male meiosis: prometaphase I with 22 bivalents (B, D); prometaphase II with 22 chromosomes (C, E).

drawings of female bivalents of both species presented by Halfer-Cervini *et al.* (1968) and Barigozzi (1974). We maintain that the analysis of meiotic cells has at least two advantages with respect to mitotic cells: the number of chromosomes is reduced to half (n = 22) and bivalents are larger than single chromosomes. From our experience, the analysis of mitotic cells, both from nauplii and males, yielded meager positive results, and chromo-

Fig. 3. Male meiosis in *Artemia* from Colorada Chica. (A) Pachytene with 22 bivalents. (B, C) Prometaphase I with 22 bivalents. (D) Prometaphase II with 22 chromosomes. (E) Male meiotic karyotype (metaphase I).

some number determination was difficult (Table 1). In the sample from Colorada Chica, for example, the diploid number could not be ascertained from somatic cells. Conversely, the meiotic analysis of adult males showed a high number of good quality prometaphase plates (Table 2).

The kinetic activity of the chromosomes of the genus *Artemia* has been a controversial subject. Stefani (1963a, b) and Stefani and Cadeddu (1967) suggested that the chromosomes of *A. salina* are holokinetic, whereas Barigozzi (1974) stated that at the time of his study it was impossible to draw any definite conclusion. Recent works neither refer to chromosome morphology, nor mention the "lack of distinct primary constrictions" (Abatzopoulos *et al.*, 1986). Our observations on cells in mitotic prometaphase and meiotic prometaphase II suggest the presence of metacentric, submetacentric, and telocentric chromosomes. Although chromosome morphology cannot be observed in all the 22 pairs, they seem to be monocentric. The pres-

ence of metacentric and telocentric chromosomes has also been suggested by Colihueque and Gajardo (1996).

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Address: Departamento di Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II, Ciudad Universitaria, 1428 Buenos Aires, Argentina. (e-mail: cohen @biolo.bg.fcen.uba.ar)