KARYOTYPES OF THE SCALLOPS *EUVOLA ZICZAC* AND *NODIPECTEN NODOSUS,* FROM THE GULF OF CARIACO, SUCRE STATE, VENEZUELA

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ABSTRACT: The chromosomes of the *Euvola ziczac* and *Nodipecten nodosus* were studied through the squash method using gills and unfertilized eggs. Diploid number of chromosome was 38 for both species. *E. ziczac* has 5 metacentric pairs, 6 submetacentric pairs, 7 subtelocentric pairs and 1 telocentric chromosome pair of. *N. nodosus* has 4 metacentric pairs, 5 submetacentric pairs, 7 subtelocentric pairs and, 3 telocentric chromosome pairs. Analysis in meiotic cells of metaphase I showed that these species fit the genetic system that has normal pairing and non-random chiasmata distribution.

RESUMEN: Los cromosomas de los pectínidos *Euvola ziczac* y *Nodipecten nodosus* fueron estudiados por el método de aplastamiento de las células, utilizando branquias y ovocitos sin fecundar. El número diploide de cromosomas fue 2n= 38 para ambas especies. *E. ziczac* tiene 5 pares metacéntricos, 6 pares submetacéntricos, 7 pares subtelocéntricos y 1 par de cromosomas telocéntrico. *N. nodosus* tiene 4 pares metacéntricos, 5 pares submetacéntricos, 7 pares subtelocéntricos y 3 pares de cromosomas telocéntricos. El análisis de células meióticas en metafase I, mostró que estas especies se ajustan al sistema genético de apareamiento normal y distribución de quiasmas no aleatorios.

INTRODUCTION

The scallops *Amusium papyraceum, Euvola ziczac* and *Nodipecten nodosus* belonging to the family Pectinidae are of economic importance in Venezuela because they have some characteristics, such as: good taste and texture and large muscle size that make them convenient for commercial explotation.

The Venezuelan Oceanographic Institute has developed many studies in order to obtain information about these species under controlled conditions: spawning and embryo development (MARÍN 1984), seasonal variations (VÉLEZ *et al.*, 1987), diet effect on survival and development (ROJAS *et al.*, 1988), spawning induction through temperature variations (VÉLEZ *et al.*, 1990, 1993), genetic variations (CORONADO *et al.*, 1991), cultive under controlled conditions (VÉLEZ & FREITES 1992), triploidy experiments (RAMÍREZ & PÉREZ 1993), development and productivity (FREITES *et al.*, 1993), heterozygosity and metabolic efficiency (ALFONSI *et al.*, 1995), consanguinity effects (BETANCOURT *et al.*, 1995).

RAMÍREZ & PÉREZ (1993) determined that the diploid chromosome number in *E. ziczac* and *N. nodosus* is 2n = 38, but their study did not report size and shape according to centromeric position of the chromosomes.

This paper studies the karyotypes of *E. ziczac* and *N. nodosus* in order to determine the chromosome similarities between these species, and to obtain basic genetic information that will contribute to develop further studies of genetic improvement.

MATERIALS AND METHODS

One hundred and twenty *Euvolac ziczac* and *Nodipecten nodosus* specimens were collected by free diving in Chacopatica, Golfo de Cariaco, Sucre State, Venezuela (10º 30' 10" N; 64º 13' 6" W), in four different months: September 1996, November 1996, and April, 1996, July, 1997.

At the laboratory, the specimens were individually conditioned and maintained in sea water at 21°C for 24 hours. *Tetraselmis chuii, Chaetoceros gracilis* and *Isochrisis aff . galbana* were used as daily food in 1:1:1 ratio (30.000 to 70.000 cells./ml).

The gill tissues were treated with 0.05% colchicine sea water for five hours, transferred to 0.9% sodiu citrate for 30 minutes and fixed in modified Carnoy solution (ethanol 95%: acetic acid, 3:1) for 20 minutes. This process was made twice and the tissues were stored in 50% acetic acid until used. The chromosomes of fixed cells were examined through the squash method and stained with 2% acetic orcein.

Unfertilized eggs were used for meiotic analysis. The spawning of *E. ziczac* and *N. nodosus* adult specimens from natural populations was performed under controlled conditions, using the methods described by VÉLEZ *et al.* (1990). When the spawning started, each specimen was isolated in plastic containers of 2 L, and unfertilized eggs were incubated with 0.2% colchicine solution in seawater during 2 h. After this, unfertilized eggs were exposed to 0,9% sodium citrate solution during 30 min, Carnoy solution and lipid solvent according to the methods described by ALFONSI *et al.* (1998). The best metaphase meiotic chromosome plates were photographed.

Thirty eight *E. ziczac* specimens were used for analysis. From such analysis, forty eight gill metaphase cells, and one hundred and thirty five meiocytes were observed. From this sample, ten gill cells and seven meiocytes were chosen and analyzed. Forty two *N. nodosus* specimens, fifty two gill metaphase cells, and one hundred and thirty six meiocytes were observed. Twelve gill cells and nine meiocytes were selected from the latter sample and analyzed.

The mitotic and meiotic metaphase chromosomes were studied under an Olympus light microscope BX 40 having a Sony Video camera connected and adapted to a computer. They were copied using a Photofinish software, and photographed with a Minolta camera X 300 S. Their images were transfered on the computer scrren. Ten mitotic methaphases in *E. ziczac* and twelve mitotic methaphase in *N. nodosus* were measured, ordered by pairs in decreasing order and analyzed statistically through a t-student test according to the method provided by LEVAN *et al.* (1964) .

The metaphase I configurations for normal diploids were tested for normal pairing and non-random chiasmata distribution, using the equations: oII = C - x, number of cells, and cII = x - (C - x), number of cells according to JACKSON & HAUBER (1994). The symbols used throughout the text and their meanigs are the following: cII = a bivalent with one chiasma, oII = a bivalent with two chiasmata, C= mean chiasmata number per cell, x = a basic chromosome number.

RESULTS AND DISCUSSION

Diploid number of chromosomes was 38 for both species of scallops. The diploid number 2n=38 was counted in all cells at mitotic metaphase. The *Euvola ziczac* chromosome complement (Fig. 1 A,B) has 5 metacentric pairs (r = 1.0 - 1.7), 6 submetacentric pairs (r = 1.7 - 3.0), 7 subtelocentric pairs (r = 3.0 - 7.0) and 1 telocentric pair (r = 7.0 - ∞). The chromosome lengths

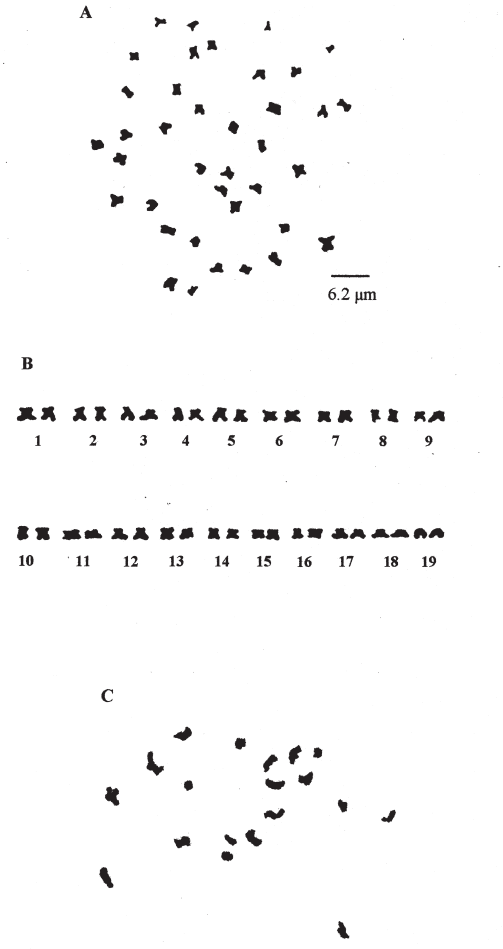

Figure 1. Metaphase of somatic chromosomes from gill cell of *Euvola ziczac* A. Diploid karyotype of *E. ziczac* B. Chromosomes at metaphase of meiosis I from unfertilized eggs of *E. ziczac* C.

ranged from 2.17 ± 0.18 mm to 0.81 ± 0.08 mm (Table 1). Metaphase I configurations showed 5 circles and 14 bivalents chains (Fig. 1 C, Tabla 3). The mean chiasmata number per cell was 24.

Table 1. Mean legths and arm ratios of chromosomes of ten metaphase plates in gill cells from *Euvola ziczac*.

Chromosome Pair	Median Length (µm)	Standard deviation	Arm ratio	Chromosome types
1	2.17	0.18	2.61	sm
2	1.78	0.08	3.06	st
3	1.47	0.09	3.01	st
4	1.45	0.03	2.67	sm
5	1.37	0.07	5.90	st
6	1.36	0.02	1.57	m
7	1.35	0.05	2.80	sm
8	1.34	0.10	4.19	st
9	1.32	0.03	3.27	st
10	1.28	0.12	1.54	m
11	1.18	0.15	2.04	sm
12	1.09	0.11	2.10	sm
13	1.06	0.18	1.59	m
14	0.95	0.20	1.26	m
15	0.92	0.17	1.50	m
16	0.91	0.14	1.73	sm
17	0.85	0.13	3.41	st
18	0.82	0.04	5.89	st
19	0.81	0.08	-	t
Total	23.48			

Nodipecten nodosus chromosome complement (Fig. 2 A,B) corresponds to 4 metacentric pairs (r=1.0 - 1.7), 5 submetacentric pairs (r =1.7 - 3.0), 7 subtelocentric pairs (r = 3.0 - 7.0), and 3 telocentric pairs (r = 7.0 - ¥). Chromosome lengths ranged from 2.51 ± 0.14 mm to 0.84 ± 0.08 mm (Table 2). Metaphase I configurations showed 4 circles and 15 bivalents chains (Fig. 2 C, Tabla 3). The mean chiasmata number per cell was 23.

The mitotic chromosomes from both species were homomorphic and isopycnotic, and different in sizes and shapes according to the centromeric position. No heteromorphic chromosome pairs were found to be responsible for sex determination. The absence of sex chromosomes in scallops has been reported by MENZEL (1968); AHMED (1973); MACKIE (1984) and VAN BRAND *et al.*, (1990).

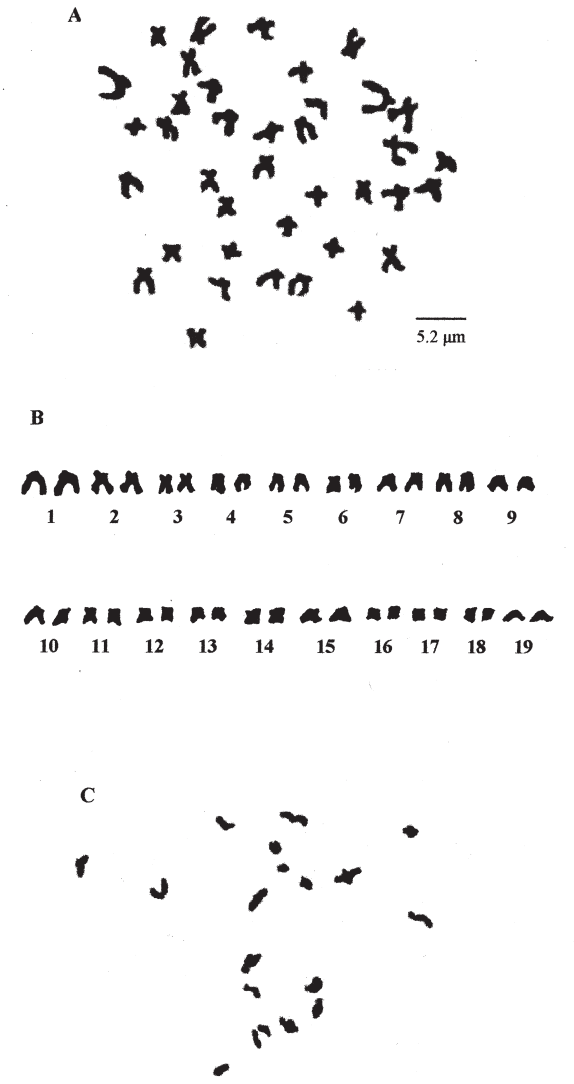

Figure 2. Metaphase of somatic chromosomes from gill cell of *Nodipecten nodosus* A. Diploid Karyotype of *N. nodosus* B. Chromosomes at metaphase of meiosis I from unfertilized eggs of *N. nodosus* C.

Significant differences were detected between the mean chromosomal length of boths scallops (ts = -2,0969; P< 0,05). The chromosome complements observed (2n= 38) in *E. ziczac* and *N. nodosus,* agreed with the number reported in other pectinidae species that include: *Pecten maximus, Chlamys varia, Ch. distorta, Ch. islandica, Ch. farreri nipponensis, Ch. farreri, Placopecten magellanicus, Pecten albicans, P. jacobaeus, Patinopecten yessoensis*, (BEAUMONT & GRUFFYD 1974; IEYAMA & VENUS 1975; RASOTTO *et al.*, 1981; KOMARU

& WADA 1985; YABU & MARU 1987). However, *Ch. opercularis* has 2n = 26 and *Argopecten irradians irradians*, *Ch. glabra, Ch. nobilis and A. purpuratus* have 2n = 32 (BEAUMONT & GRUFFYD 1974; WADA 1978; RASOTTO *et al.*, 1981; KOMARU & WADA 1985; VAN BRAND *et al.*, 1990).

Table 2. Mean legths and arm ratios of chromosomes of twelve metaphase plates in gill cells from *Nodipecten nodosus*.

Chromosome Pair	Median Length (μm)	Standard deviation	Arm ratio	Chromosome types
1	2.51	0.14	-	t
2	2.06	0.10	3.75	st
3	2.03	0.08	2.17	sm
4	1.78	0.09	-	t
5	1.71	0.14	3.34	st
6	1.68	0.19	3.32	st
7	1.67	0.09	2.38	sm
8	1.67	0.14	3.32	st
9	1.66	0.04	3.01	st
10	1.60	0.20	4.52	st
11	1.40	0.06	1.86	sm
12	1.29	0.11	1.98	sm
13	1.27	0.14	2.08	sm
14	1.26	0.15	1.28	m
15	1.24	0.13	3.58	st
16	1.05	0.17	1.26	m
17	0.98	0.12	1.19	m
18	0.87	0.09	1.36	m
19	0.84	0.08	-	
Total	28.57			

Both studied scallop species had the same chromosome types and fitted a genetic system having a normal pairing and a normal non-random chiasmata distribution (Table 3). There is a minimum of one chiasma and a maximum of two chiasmata per bivalent, and the bivalents are essentially equal in crossover frequency. However, there are some chromosome differences, such as the following: a) cytological measurement indicating that *N. nodosus* chromosomes were larger than *E. ziczac,* b) *E. ziczac* has 5 metacentric and 6 submetacentric chromosome pairs while *N. nodosus* has 4 metacentric and 5 submetacentric chromosome pairs, c) *E. ziczac* has 1 telocentric chromosome pair while *N. nodosus* has 3 telocentric chromosome pairs. These species are normally found in the same habitats and no variation in chromosome number and types was observed in all mitotic and meiotic cells analyzed, which may indicate that chromosome isolating barriers are very effective.

Studies of the karyotypes in *Pecten maximus*, *P. albicans, Chlamys farreri and Patinopecten yessoensis* have indicated the presence of metacentric, submetacentric and telocentric chromosomes with a high number of telocentric chromosomes (BEAUMONT & GRUFFYD 1974; KOMARU & WADA 1985). These chromosomes were larger than *E. ziczac* and *N. nodosus* chromosomes.

Assuming that karyotypes in *P. maximus, P. albicans, Patinopecten yessoensis, Argopecten irradians irradians, Ch. nobilis and A. purpuratus* have been originated from an ancestral species with 2n = 38 chromosomes, the probable direction of evolution might have started from an ancestral form similar to *P. maximus* or *P. albicans* to *P. yessoensis*, *E. ziczac* and *N. nodosus* in one evolutionary line, where the telocentric chromosome centromeres have changed location through pericentric inversion or centric transposition resulting in metacentric or submetacentric chromosomes shorter than the ancestral species (WHITE 1973; AYALA & KIGER 1980). A second evolutionary line started from a form with 19 telocentric chromosome pairs, leading to *A. irradians irradians*, *Ch. nobilis* and *A. purpuratus,* where a Robertsonian centric fusion has taken place (AYALA & KIGER 1980; KOMARU & WADA 1985). Therefore, the modal number of 2n = 38 in the Pectinidae family has changed in some species during the evolution of the group. Chromosome evolution depends on pericentric inversions, centric transpositions or chromosome fusion /fission, but these events must involve the centromere.

Table 3. Comparison of observed (Obs.) and expected (Exp) oII and cII at metaphase I in 7 cells of *Euvola ziczac* and 9 cells of *Nodipecten nodosus*

	oII		cII		
Species	Obs	Exp	Obs	Exp	X^2
E.ziczac	35	35	89	98	0
N. nodosus	36	36	135	135	0

CONCLUSIONS

Euvola ziczac and *Nodipecten nodosus* have a chromosome complement of 2N=38. The *E. ziczac* chromosome lenghts ranged from 0,81µm to 2,17 µm and *N. nodosus* ranged from 0,84 µm to 2,51 µm. The chromosomes have normal pairing and non-random chiasmata distribution. Pericentric inversions and centric transpositions may have taken place during the *E. ziczac* and *N. nodosus* chromosome evolution.

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