Cytogenetics of the razor clam *Solen marginatus* (Mollusca: Bivalvia: Solenidae)*

J. Fernández-Tajes, A. González-Tizón, A. Martínez-Lage and J.Méndez†

Dpto. de Biología Celular y Molecular, Universidade da Coruña, La Coruña (Spain)

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Abstract

The razor clam *Solen marginatus* has a diploid chromosome number of 38. The karyotype consists of one metacentric/submetacentric, three submetacentric/metacentric, five submetacentric, one submetacentric/subtelocentric, one subtelocentric/submetacentric, six subtelocentric and two telocentric chromosome pairs. Staining with chromomycin A_3 revealed bright positive bands subcentromerically in the long arms of one medium-sized subtelocentric pair, while DAPI staining showed uniform fluorescence in all chromosomes of the complement. Fluorescence in situ hybridization using an 18S-5.8S-28S rDNA probe locates these loci at the subcentromeric region of one subtelocentric pair and at the subtelomeric region of another subtelocentric pair.

Introduction

In recent years, the number of cytogenetic studies on bivalves has remarkably increased. Since 1992 karyotypes of about 65 species have been studied by banding techniques and fluorescence in situ hybridization (FISH), although most of them are limited to a few families, such as Mytilidae, Ostreidae, Pectinidae, Cardiidae and Veneridae (Thiriot-Quievreux, 2002). Other families of bivalves, such as Solenidae, commonly known as razor clams, are poorly and scarcely analyzed. To our knowledge only the karyotype (chromosome number and morphology) of a Chinese population of *Solen constrictus* has been described (Wang et al., 1998). Solenids are marine species and inhabit fine sand, silt or mud biotopes. They are highly adapted for life in these habitats owing to their capacity for rapid vertical burrowing into sand. Adaptative features include the characteristic shape of their shell which is elongated and laterally compressed and has almost semicylindrical valves. For these reasons razor clams are highly specialized organisms and very successful in their habitats (Yonge, 1952). Solenidae have been known since the early Eocene and presently 60–65 living species exist. Usually they colonize continental waters and few solenids have been recorded from oceanic islands (von Cosel, 1990).

Razor clams are commercially exploited in several european countries such as Spain, Portugal, Italy or Ireland. Three commercialized razor clam species are distributed along the Galician coasts (northwest Spain). Two of them, *Ensis arcuatus* and *Ensis siliqua* (family Pharidae), have been analyzed by González-Tizón et al. (in preparation). The third species, *Solen marginatus* (family Solenidae), is analyzed in the present work in an attempt to gain knowledge about its cytogenetics as an initial step to characterize and evaluate natural populations of this bivalve.

Materials and methods

Sample collection and chromosome preparation

Individuals of *Solen marginatus* were collected from Boiro (Ría de Arousa, NW Spain). In the laboratory animals were fed with a suspension of microalgae (*Isochrisis galbana* and *Tetraselmis* sp.) for 10–15 days. Metaphases were obtained after treatment with colchicine solution solution (0.005%) for 6–8 h. Gills were dissected and treated twice with 0.56% KCl solution for 15 min. After fixation in ethanol:glacial acetic acid (3:1) cells were dissociated in 45% acetic acid and dropped onto slides heated at 43°C. Metaphases were stained with 4% Giemsa in phosphate buffer pH 6.8 and photographed with a Leica DM-RXA microscope.

Determination of DNA content

For DNA content determination nuclei were stained using the method described by Feulgen and Rossenbeck (1924) and the microdensitometric measurements were made using Leica image analysis software. Twelve individuals and 20 nuclei from each individual were measured. The mussel *Mytilus galloprovincialis* which possesses 3.84 pg of DNA (Rodríguez-Juiz et al., 1996) was used as a standard. Integrated optical density values of the samples tested were transformed to picograms using an arithmetic conversion.

Karyotyping

Chromosome measurements were made using the Leica Chantal image analysis software system (see González-Tizón et al., 2000). Twelve metaphases from 12 individuals were analyzed. Mean length of the chromosome arms and the mean value for their total chromosome length were calculated for each chromosome pair. The relative length (100× chromosome length/total haploid length), the centromeric index (100× length of short arm/total chromosome length), the mean value and the standard error (standard deviation/number of individuals) 1/2 of the relative lengths and centromeric indices were also calculated. Karyotypes were arranged by decreasing chromosome size and classified according to the centromeric index, following the nomenclature of Levan et al. (1964). Chromosome number was determined after evaluation of 250 metaphases from 25 individuals.

Fluorochrome staining

Chromomycin A_3 (CA_3) and DAPI staining were applied following the methods of Schweizer (1976, 1980). Metaphases were treated with 0.5 mg/ml of CA_3 in stock solution (5 ml of McIlvainesøs buffer pH 7.0, 0.1 ml of 50 mM $MgCl_2$ and 4.9 ml of Milli-Q water) for 1–2 h and then the slides were washed in distilled water and air-dried. DAPI staining was carried out by placing 35 µl of DAPI solution (0.33 µl g/ml) on slides for 20–30 min. After washing with distilled water slides were air-dried. 150 metaphases from 13 individuals were analyzed after fluorochrome staining.

Fluorescence in situ hybridization (FISH)

FISH was performed as described by González-Tizón et al. (2000) using pDm 238 from *Drosophila melaelanogaster* containing the repeat unit 18S-5.8S-28S rDNA (Roiha et al., 1981) as a probe. It was labeled with digoxigenin-11-dUTP using a nick translation kit (Roche) according to manufacturer's instructions. Slides were counterstained with propidium iodide (50 ng/µl antifade), visualized and photographed using a Leica DM-RXA microscope equipped with the appropriate filters. Photographs were taken on Kodak Ektachrome 400 ASA films.

Results and discussion

The analysis of the Feulgen-stained nuclei revealed that *Solen marginatus* has a diploid DNA content of 5.12 pg. This value is higher than that of other razor clams. Hinegardner (1974) obtained a DNA content of 3.00 pg and 3.40 pg in *Ensis directus* and *Solen viridis*, respectively and González-Tizón et al. (in preparation) obtained 4.00 pg and 3.85 pg in *E. arcuatus* and *E. siliqua* , respectively. Intra- and interspecific variation of genome size has been reported within many groups of plants and animals, including related species (Gregory and Herbert, 1999; Gregory et al., 2000) and even conspecific populations (Lockwood and Bickham, 1992). Many theories have been proposed to explain the variation in genome size or DNA content. Ohno (1972) suggested that the majority of the genome consists of non-coding DNA or "junk DNA" which is not subjected to constraining selection and, subsequently, genomes tend to increase in size through the evolution process. Doolitle and Sapienza (1980), among other authors, suggested the existence of a dynamic evolutionary process within the non-coding DNA. This process consists of the expansion of different genome fractions as transposable elements, bacterial plasmids or retroviruses, competing amongst each other for maximum representation. Such expansion would increase the genome size. Other theories suppose a coevolution between genome size and cell volume (Cavalier-Smith, 1978), such that the C-value of an organism results from selection for a given nuclear volume. Despite these theories the C-value paradox yet remains unclear.

The chromosome number of *S. marginatus* is 2n = 38 (Figs. 1a and 2). Within the bivalve class this is the most frequent chromosome number (Thiriot-Quievreux, 2002). It has also been reported in different species belonging to the families Nuculidae, Arcidae, Pectinidae, Unionidae, Cardiidae, Mactridae, Tellinidae, Psammobiidae, Corbiculidae, Veneridae (for review see Thiriot-Quievreux, 2002) and Donacidae (Martínez et al., 2002). However, the family Solenidae has not been investigated so far and only the number and karyotype of *Solen constrictus* from a Chinese population has been reported by Wang et al. (1998).

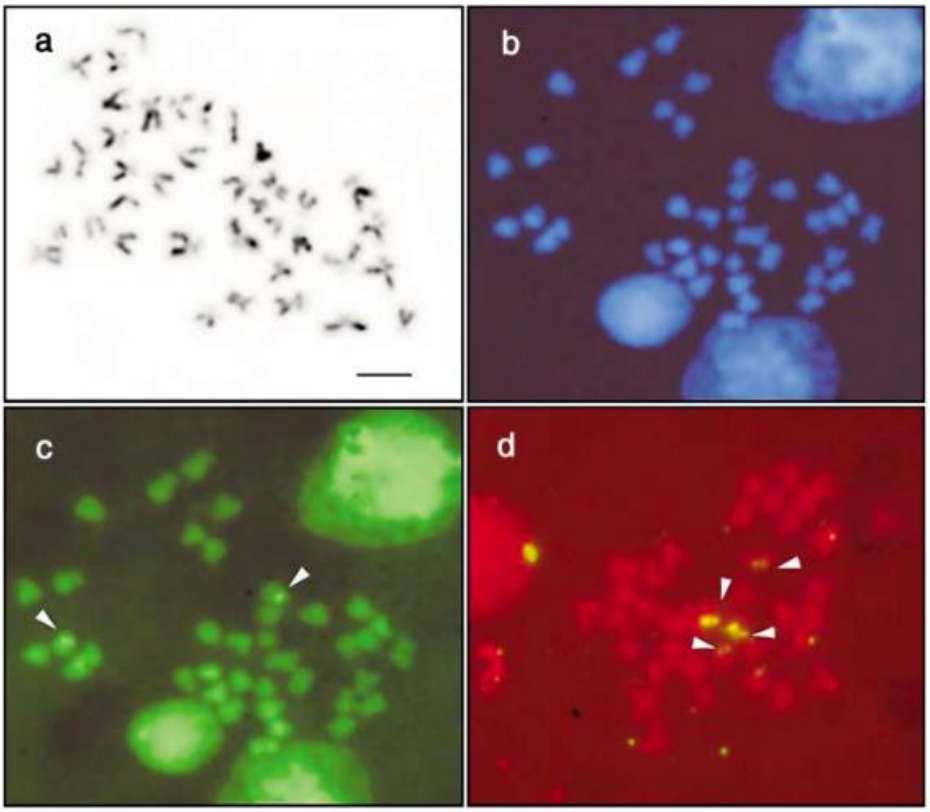

Figure 1. Metaphases of *Solen marginatus* after (a) Giemsa staining, (b) DAPI staining, (c) Chromomycin A_3 staining and (d) FISH with an 18S-5.8S-28S rDNA probe. Bar = 10 µm.

The relative chromosome lengths and the centromeric index values of *S. marginatus* are shown in Table 1. The relative lengths vary from 7.03 to 3.62 and the karyotype shows one metacentric/submetacentric chromosome pair (No. 5), three submetacentric/metacentric (Nos. 1, 8, and 18), five submetacentric (Nos. 3, 10, 12, 13, and 16), one submetacentric/subtelocentric (No. 15), one subtelocentric/submetacentric (No. 4), six subtelocentric (Nos. 2, 6, 7, 9, 11, and 14) and two telocentric chromosome pairs (Nos. 17 and 19). This karyotype is clearly different from that of *S. constrictus* which shows 15 metacentric, three submetacentric and one subtelocentric pair (Wang et al., 1998). Such differences could be due to chromosome rearrangements occurring in the evolution of these species or to differences in chromosome condensation.

Table 1. Chromosome measurements and classification in *Solen marginatus*

C.N.[a]	C.I.[b]	R.L.[c]	Class[d]
1	7.03 ± 0.21	34.24 ± 1.84	sm/m
2	6.59 ± 0.15	21.40 ± 2.31	st
3	6.45 ± 0.12	29.16 ± 1.96	sm
4	6.07 ± 0.06	24.41 ± 1.08	st/sm
5	5.83 ± 0.06	35.39 ± 1.71	m/sm
6	5.81 ± 0.09	22.62 ± 1.91	st
7	5.40 ± 0.02	20.39 ± 1.34	st
8	5.39 ± 0.10	33.83 ± 2.07	sm/m
9	5.31 ± 0.03	23.13 ± 1.15	st
10	5.28 ± 0.10	30.54 ± 1.99	sm
11	5.14 ± 0.07	22.19 ± 2.41	st
12	4.98 ± 0.06	29.14 ± 1.51	sm
13	4.93 ± 0.12	27.87 ± 1.50	sm
14	4.84 ± 0.10	18.75 ± 2.71	st
15	4.80 ± 0.10	25.98 ± 1.12	sm/st
16	4.68 ± 0.10	32.50 ± 1.56	sm
17	3.94 ± 0.13	3.94 ± 2.60	t
18	3.92 ± 0.19	33.67 ± 1.84	sm/m
19	3.62 ± 0.12	4.37 ± 3.02	t

[a] C.N. – chromosome number
[b] C.I. – centromeric index
[c] R.L. – relative length
[d] Class – classification: m- metacentric; sm- submetacentric; t- telocentric; st- subtelocentric.

To date chromosomes of five species of Mytilidae (Martínez-Lage et al., 1994, 1995; Torreiro et al., 1999; Vitturi et al., 2000), one species of Donacidae (Martínez et al., 2002) and two species of Pharidae (González-Tizón et al., in preparation) have been stained using CA_3 and DAPI fluorochromes. In *S. marginatus* the number of CA_3 bands varied from two to four; two of them are located subcentromerically in the long arms of the medium-sized subtelocentric pair 9 (Figs. 1b and 2). The other two bands fluoresce only weakly and are located subtelomerically in a subtelocentric pair. In some cases these bands were not even visible.

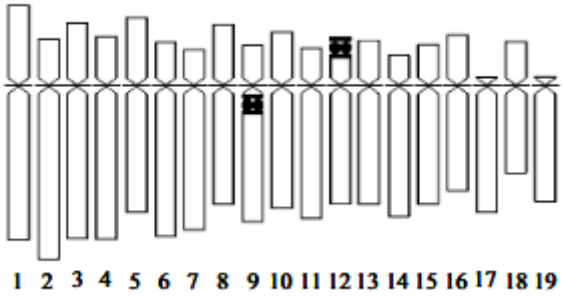

Figure 2. Idiogram of *Solen marginatus*. Black dots represent CA_3-positive bands; black refill represents the location of the 18S-5.8S-28S ribosomal loci.

In comparison with the other bivalves, *S. marginatus* shows similar amounts of GC-rich CA_3-positive eterochromatin to the five mytilids analyzed, but a very low amount compared with the donacid (*Donax trunculus*) and pharid species (*Ensis arcuatus* and *E. siliqua*). Similar to the eight bivalves mentioned above, staining with DAPI reveals uniform fluorescence in all chromosomes of the complement of *S. marginatus* (Fig. 1c).

Fluorescence in situ hybridization using an 18S-5.8S-28S rDNA probe has been applied to 14 different species of bivalve: five Mytilidae (Insua and Méndez, 1998; Torreiro et al., 1999; González-Tizón et al., 2000; Vitturi et al., 2000), two Ostreidae (Zhang et al., 1999; Xu et al., 2001), one Pectinidae (Insua et al., 1998), one Cardiidae (Insua et al., 1999), one Tellinidae (González-Tizón et al., 2000), one Psammobiidae (González-Tizón et al., 2000), one Donacidae (Martínez et al., 2002) and two Pharidae (González-Tizón et al., in preparation). In all of these species most ribosomal loci locate at telomeric, and less frequently, at subtelomeric regions. *S. marginatus* shows these major ribosomal loci at a subcentromeric region in the subtelocentric pair No. 9 (bright fluorescence) and at a subtelomeric region on the subtelocentric No. 12 (weak fluorescence) (Figs. 1d and 2). As with CA_3 bands, a third chromosome pair (telocentric) appears to be weakly labeled after FISH, and in some cases these signals could not be observed. As pointed out previously by several authors in many plant and animal species, this phenomenon could be due to the existence of a low number of rDNA copies on this pair. Furthermore, in *S. marginatus* the location of these loci at the subcentromeric region of chromosome No. 9 and at the subtelomeric region of pair No. 12 are coincident with CA_3-positive bands on these same chromosome pairs, which confirms that the rDNA genes are GC-rich.

In conclusion, this work describes for the first time the cytogenetics of the commercially important razor clam *S. marginatus*, and the results contribute to the number of bivalve species analyzed to date.

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References

Cavalier-Smith T: Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate and the solution of the C-value paradox. *J Cell Sci 34*:247–278 (1978).

Doolitle WF, Sapienza C: Selfish genes, the phenotype paradigm and genome evolution. *Nature 284*:617–618 (1980).

Feulgen R, Rossenbeck H: Mikroskopisch-chemischer Nachweis einer Nucleinsäure vom Typus der Thymonucleinsäure und die darauf beruhende selektive Färbung von Zellkernen in mikroskopischen Präparaten. *Z physiol Chem 135*:203–248 (1924).

González-Tizón A, Martínez-Lage A, Rego I, Ausio J, Méndez J: DNA content, karyotype and chromosomal location of 18S-5.8S-28S ribosomal loci in some species of bivalve mollusc from the Pacific Canadian coast. *Genome 43*:409–411 (2000).

Gregory TR, Herbert PDN: The modulation of DNA content: proximate causes and ultimate consequences. *Genome Res 9*:317–324 (1999).

Gregory TR, Herbert PDN, Kolasa J: Evolutionary implications of the relationship between genome size and body size in flatworms and copepods. *Heredity 84*:201–208 (2000).

Hinegardner R: Cellular DNA content of the Mollusca. *Comp Biochem Physiol 47*:447–460 (1974).

Insua A, Méndez J: Physical mapping and activity of ribosomal RNA genes in mussel *Mytilus galloprovincialis*. *Hereditas 128*:189–194 (1998).

Insua A, López-Piñón MJ, Méndez J: Characterization of *Aequipecten opercularis* (Bivalvia: Pectinidae) chromosomes by different staining techniques and fluorescent in situ hybridization. *Genes Genet Syst 73*:193–200 (1998).

Insua A, Freire R, Méndez J: The 5S rDNA of the bivalve *Cerastoderma edule*: nucleotide sequence of the repeat unit and chromosomal location relative to 18S-28S rDNA. *Genet Sel Evol 31*:509–518 (1999).

Levan A, Fredga K, Sandberg AA: Nomenclature for centromeric position on chromosomes. *Hereditas 52*:201–220 (1964).

Lockwood SF, Bickham JW: Genome size in Beaufort sea coastal assemblages of Arctic ciscoes. *T Am Fish Soc* 121:13–20 (1992).

Martínez A, Mariñas L, González-Tizón A, Méndez J: Cytogenetic characterization of *Donax trunculus* (Bivalvia: Donacidae) by means of karyotyping, fluorochrome banding and fluorescent in situ hybridization. *J Moll Stud 68*:393–396 (2002).

Martínez-Lage A, González-Tizón A, Méndez J: Characterization of different chromatin types in *Mytilus galloprovincialis* L. after C-banding, fluorochrome and restriction endonuclease treatments. *Heredity 72*:242–249 (1994).

Martínez-Lage A, González-Tizón A, Méndez J: Chromosomal markers in three species of the genus *Mytilus* (Mollusca: Bivalvia). *Heredity 74*:369–375 (1995).

Ohno S: So much "junk" DNA in our genome, *in* Smith HH (ed): *Evolution of Genetic Systems*, pp 366–370 (Gordon Breac, New York 1972).

Rodríguez-Juiz AM, Torrado M, Méndez J: Genome-size variation in bivalve molluscs determined by flow cytometry. *Mar Biol 126*:489–497 (1996).

Roiha H, Miller JR, Woods LC, Glower DM: Arrangements and rearrangements of sequences flanking the two types of rDNA insertion in *D. melanogaster*. *Nature 290*:749–753 (1981).

Schweizer D: Reverse fluorescent chromosome banding with chromomycin and DAPI. *Chromosoma 58*:307–324 (1976).

Schweizer D: Simultaneous fluorescent staining of R-bands and specific heterochromatic regions (DA-DAPI bands) in human chromosomes. *Cytogenet Cell Genet 27*:190–193 (1980).

Thiriot-Quievreux C: Review of the literature on bivalve cytogenetics in the last ten years. *Cah Biol Mar 43*:17–26 (2002).

Torreiro A, Martínez-Expósito MJ, Trucco MI, Pasantes JJ: Cytogenetics in *Brachidontes rodriguezi* d'Orb (Bivalvia, Mytilidae). *Chrom Res 7*:49–55 (1999).

Vitturi R, Gianguzza P, Colomba MS, Riggio S: Cytogenetic characterization of *Brachidontes pharaonis* (Fisher P, 1870): karyotype, banding and fluorescent *in situ* hybridization (FISH) (Mollusca: Bivalvia: Mytilidae). *Ophelia 52*:213–220 (2000).

von Cosel R: An introduction to the razor shells (Bivalvia: Solenacea), in Morton B (ed): *The Bivalvia-Proceedings of a Memorial sympsium in Honour of Sir Charles Maurice Yonge*, pp 283–305 (Hong Kong University Press, Hong Kong 1990).

Wang J, Zhao X, Zhou L, Xiang J: Karyotypes of three species of marine bivalve molluscs. *Acta Ocean Sin 20*:102–107 (1998).

Xu Z, Guo XM, Gaffney PM, Pierce JC: Chromosomal location of the major ribosomal RNA genes in *Crassostrea virginica* and *Crassostrea gigas*. *Veliger 44*:79–83 (2001).

Yonge CM: Observations on *Siliqua patula* Dixon and on evolution within the Solenidae. *Univ Calif Publ Zool 55*:421–438 (1952).

Zhang Q, Yu G, Cooper RK, Tiersch TR: Chromosomal location by fluorescence *in situ* hybridization of the 28S ribosomal RNA gene of the Eastern oyster. *J Shell Res 18*:431–435 (1999).

[†] fina@udc.es