



Genome sizes and karyotypes in the razor clams *Ensis arcuatus* (Jeffreys, 1865) and *E. siliqua* (Linnaeus, 1758)

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Abstract: The razor clams *Ensis arcuatus* and *E. siliqua* show a diploid DNA content of 3.85 ± 0.049 pg and 4.00 ± 0.050 pg, respectively. Both have a diploid chromosome number of 38 although their karyotypes show remarkable differences. The karyotype of *E. arcuatus* consists of 4 metacentric, 1 metacentric-submetacentric, 7 submetacentric and 7 telocentric chromosome pairs, whereas that of *E. siliqua* possesses 3 metacentric, 7 submetacentric and 9 telocentric pairs. *In situ* hybridization using an 18S-5.8S-28S rDNA probe located this ribosomal locus on one chromosome pair for both species. Results demonstrate that large differences exist between them, probably caused by chromosome rearrangements along evolution of these two species, and increase the number of studies on bivalve cytogenetics.

Résumé : Taille du génome et caryotype chez les couteaux *Ensis arcuatus* (Jeffreys, 1865) et *E. siliqua* (L., 1758). Le contenu de l'ADN diploïde des couteaux *Ensis arcuatus* et *E. siliqua* est de $3,85 \pm 0,049$ pg et $4,00 \pm 0,050$ pg, respectivement. Ils ont tous les deux un nombre de chromosomes diploïdes de 38 bien que leurs caryotypes présentent des différences. Le caryotype d'*E. arcuatus* est constitué de 4 paires de chromosomes métacentriques, 1 métacentrique-submétacentrique, 7 submétacentriques et 7 télacentriques, alors que celui d'*E. siliqua* possède 3 paires métacentriques, 7 submétacentriques et 9 télacentriques. Une hybridation *in situ* réalisée avec une sonde ADNr 18S-5,8S-28S a permis de localiser ce locus ribosomal sur une paire de chromosomes pour les deux espèces. Les résultats démontrent que de grandes différences existent entre elles, probablement provoqués par des réarrangements de chromosomes au cours de l'évolution de ces deux espèces, ils renforcent les études cytogénétiques réalisées sur les mollusques bivalves.

Keywords: Bivalves • Razor clams • Karyotype • Ribosomal locus • FISH (Fluorescence *in situ* hybridization)

Introduction

Ensis razor clams are highly specialized and successful bivalve species that inhabit fine sand, silt or mud bottoms. These animals belong to subclass Heterodonta, order Veneroidea, family Pharidae, which includes 50-57 living species (von Cosel, 1990) distributed in eight genera: *Ensis*, *Phaxas*, *Cultellus*, *Pharus*, *Siliqua*, *Sinonovacula*, *Pharella* and *Orbicularia* (Von Cosel, 1990). *Ensis* lives mainly in warm to cold-temperate waters, with only three known tropical species (von Cosel, 1990). In Europe, seven *Ensis* species are described: *E. arcuatus* (Jeffreys, 1865), *E. siliqua* (Linnaeus, 1758), *E. ensis* (Linnaeus, 1758), *E. magnus* (Schumacher, 1817), *E. phaxoides* (Van Urk, 1964), *E. sicula* (Van Urk, 1964) and *E. minor* (Chenu, 1843).

Ensis species are not under such a great commercial exploitation, although in the European market the importation rates reached a quote of 550 million Euros in 2004, 47% of which belongs to importation from Spain. Two of the most commercially important species are *Ensis arcuatus* (Jeffreys, 1865) and *E. siliqua* (Linnaeus, 1758), which distribute along Iberian Peninsula.

No cytogenetic study was made on these closely related species, although there are some references on chromosome data of taxa close to Pharidae. So, chromosome number has been reported in three species belonging to family Tellinidae: *Macoma balthica* and *Tellina tenuis* (Cornet & Soulard, 1990) and *Macoma nasuta* (González-Tizón et al., 2000), two species belonging to family Solenidae: *Solen constrictus* (Wang et al., 1998) and *S. marginatus* (Fernández-Tajes et al., 2003), and one belonging to family Psammobiidae: *Nuttallia nuttallii* (González-Tizón et al., 2000). A considerable number of cytogenetic studies have been published in other species included in order Veneroidea, basically on chromosome number, while those using conventional banding or fluorescence *in situ* hybridization (FISH) are scarce. In this sense, the vertebrate telomeric repeat (TTAGGG)_n and major ribosomal RNA have been assigned to different clam species (Insua et al., 1999; González-Tizón et al., 2000; Wang & Guo, 2001 & 2007; Martínez et al., 2002; Plohl et al., 2002; Fernández-Tajes et al., 2003; Hurtado & Pasantes, 2005). Very recently, molecular studies appeared in *E. siliqua* using random amplified DNA markers (Fernández-Tajes et al., 2007) and microsatellites (Varela et al., 2007).

Comparative cytogenetic analyses constitute valuable information to investigate systematic, phylogenetic and evolutionary processes of species, as well as to provide insights in the intraspecific variability and possible mechanisms of speciation. The central aim of this work was to obtain, describe and analyze the chromosome

complement of *Ensis arcuatus* and *E. siliqua* and to locate the major ribosomal locus by means of fluorescent *in situ* hybridization, to gain information on cytogenetics of bivalve species.

Material and Methods

Individuals of *E. arcuatus* were collected from Rodas de Cíes (42° 13' N, 8° 54' W) and those of *E. siliqua* from Sardiñeiro (42° 55' N, 9° 15' W), both on the northwest coast of Spain. In the laboratory, animals were fed with a suspension of *Isochrysis galbana* and *Tetraselmis sp.* microalgae for 10-15 days. Specimens were identified according to morphological characters (van Urk, 1964).

Chromosome preparation, determination of DNA content, and fluorescence *in situ* hybridization were performed as described by Fernández-Tajes et al. (2003). For DNA measurements, Feulgen-stained nuclei from five individuals from each species and 40 nuclei from each individual were used. For karyotyping, 12 metaphases from 12 individuals from each species were analyzed. For *in situ* hybridization we analyzed 10 metaphases from eight individuals of *E. arcuatus* and ten individuals of *E. siliqua*.

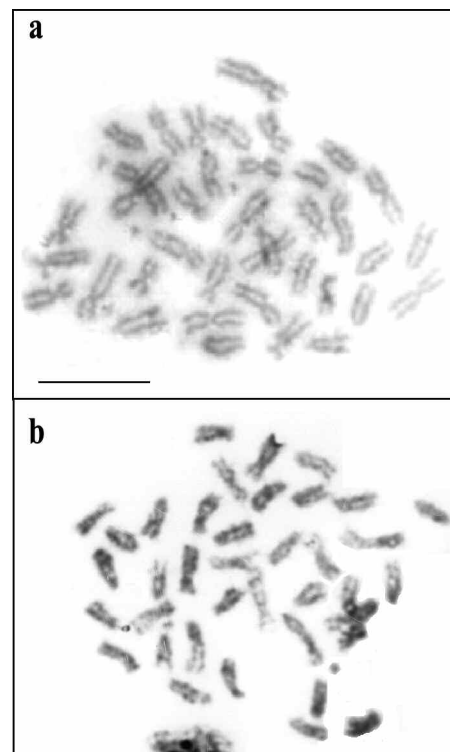


Figure 1. Metaphases of (a) *Ensis arcuatus* and (b) *E. siliqua*. Bar = 10 μ m.

Figure 1. Métaphases de (a) *Ensis arcuatus* et (b) *E. siliqua*. Echelle = 10 μ m.

Results

DNA content and karyotypes

Analysis of Feulgen-stained nuclei revealed similar C-values for both species: *E. arcuatus* showed a diploid DNA content of 3.85 ± 0.049 pg and that of *E. siliqua* was 4.00 ± 0.050 pg.

The diploid chromosome number for each species is 38 (Fig. 1a, b). In *E. arcuatus*, relative length varies from 3.82 to 7.15 (Table 1) and the karyotype consists of 4 metacentric chromosome pairs, 1 metacentric-submetacentric, 7 submetacentric and 7 telocentric (Fig. 2). In *E. siliqua*, relative length varies from 3.71 to 7.07 (Table 1) and the karyotype consists of 3 metacentric chromosome pairs, 7 submetacentric and 9 telocentric (Fig. 3).

Fluorescence in situ hybridization (FISH)

Fluorescence *in situ* hybridization using an 18S-5.8S-28S rDNA probe revealed the major ribosomal locus at the sub-centromeric region of one submetacentric chromosome pair in *E. arcuatus* (Fig. 4a). In *E. siliqua* this locus is located on one submetacentric pair at subtelomeric region (Fig. 4b), showing the FISH signals different intensity between the homologous chromosomes.

Discussion

The C-values obtained for *E. arcuatus* and *E. siliqua* are included in the range obtained for other Heterodonta species analysed (Hinegardner, 1974; Cavalier-Smith, 1978; Rodríguez-Juiz et al., 1996; González-Tizón et al., 2000), and they are very similar to those described for some bivalves belonging to families close to Pharidae (González-Tizón et al., 2000; Fernández-Tajes et al., 2003), and higher than those reported by Hinegardner (1974) in the American *Ensis directus* (3.00 pg). The differences observed in DNA content among congeneric species can be due to the different methodologies used by the authors or to quantitative variations in the amounts of repetitive DNA/heterochromatin (Redi et al., 2001). In this sense, differences in the quantity, position and properties of heterochromatin among related species are very common in animals and plants (Sumner, 2003, p. 200) and, furthermore, a low correlation exists between nuclear DNA content and chromosome number (Méndez et al., 2001). So, for example, the DNA content of *Ostrea edulis* (with a diploid chromosome number of 20) is 2.33 pg, almost identical to that of *Spisula solida* (2.32 pg) with $2n = 36$ chromosomes.

This study provides the first report about cytogenetics on *E. arcuatus* and *E. siliqua*. Both species have the same

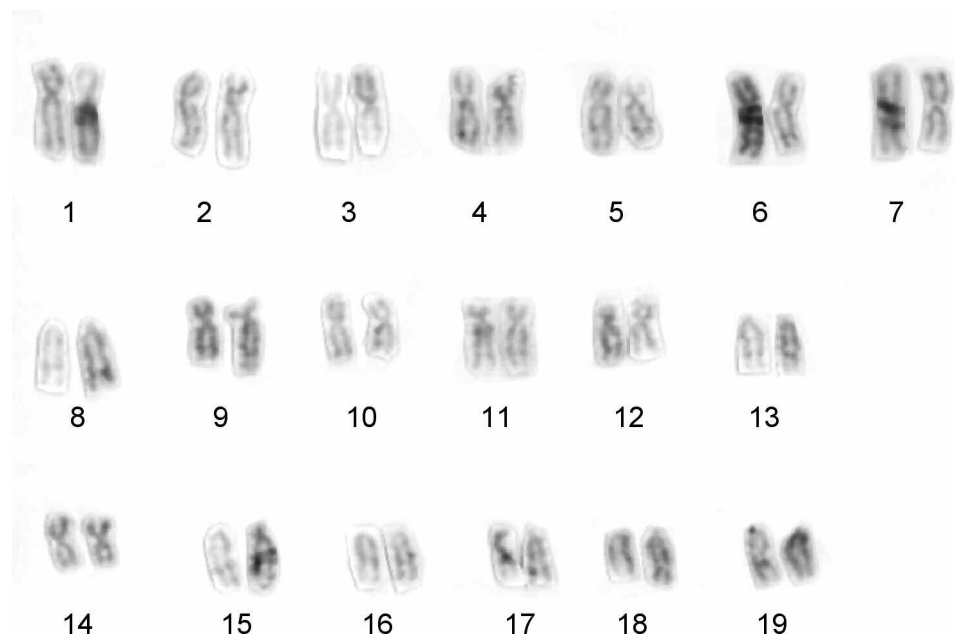


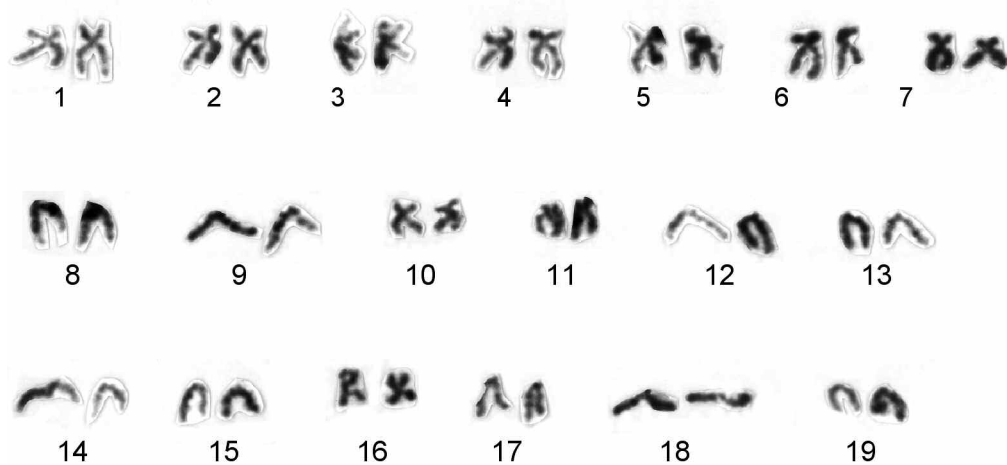
Figure 2. Karyotype of *Ensis arcuatus* ($2n = 38$) with seven telocentric chromosome pairs (# 8, 13, 15, 16, 17, 18 and 19).

Figure 2. Caryotype d'*Ensis arcuatus* ($2n = 38$), dont sept paires de chromosomes télocentriques (n° 8, 13, 15, 16, 17, 18 & 19).

Table 1. Chromosome measurements and classification.**Tableau 1.** Mesures et classification des chromosomes.

C.N.	Rel. Lgth.	<i>E. arcuatus</i>		Class.	<i>E. siliqua</i>	
		Cen. Ind.	Class.		Rel. Lgth.	Cen. Ind.
1	7.15 ± 0.52	32.04 ± 0.77	sm	7.07 ± 1.10	34.71 ± 1.10	sm
2	6.87 ± 0.85	38.48 ± 2.03	m-sm	6.53 ± 1.02	45.52 ± 0.46	m
3	6.63 ± 0.19	43.21 ± 1.02	m	6.49 ± 1.03	35.74 ± 1.15	sm
4	6.60 ± 0.27	32.77 ± 1.26	sm	6.46 ± 1.02	33.05 ± 0.70	sm
5	5.96 ± 0.17	33.52 ± 1.40	sm	5.93 ± 0.93	45.51 ± 0.67	m
6	5.82 ± 0.14	32.76 ± 0.80	sm	5.75 ± 0.89	32.75 ± 0.73	sm
7	5.77 ± 0.21	45.73 ± 0.61	m	5.51 ± 0.91	33.47 ± 1.16	sm
8	5.20 ± 0.14	0.37 ± 0.12	t	5.47 ± 0.85	0.45 ± 0.30	t
9	5.16 ± 0.10	30.14 ± 1.53	sm	5.16 ± 0.80	0.34 ± 0.27	t
10	5.02 ± 0.24	44.13 ± 0.97	m	5.14 ± 0.81	30.03 ± 0.64	sm
11	4.99 ± 0.14	30.82 ± 0.81	sm	5.02 ± 0.79	26.52 ± 0.95	sm
12	4.95 ± 0.11	35.24 ± 0.64	sm	4.99 ± 0.77	0.27 ± 0.10	t
13	4.86 ± 0.14	0.04 ± 0.08	t	4.82 ± 0.75	0.23 ± 0.08	t
14	4.69 ± 0.17	42.14 ± 1.41	m	4.65 ± 0.72	0.12 ± 0.07	t
15	4.64 ± 0.15	0.77 ± 0.34	t	4.53 ± 0.70	0.56 ± 0.33	t
16	4.41 ± 0.13	0.22 ± 0.14	t	4.50 ± 0.70	44.62 ± 1.13	m
17	4.24 ± 0.14	0.21 ± 0.17	t	4.28 ± 0.66	0.28 ± 0.19	t
18	4.14 ± 0.13	0.52 ± 0.28	t	4.00 ± 0.62	0.40 ± 0.14	t
19	3.82 ± 0.12	0.23 ± 0.11	t	3.71 ± 0.58	0.17 ± 0.08	t

C.N.: Chromosome number; Rel. Lgth.: Relative length; Cen. Ind.: Centromeric Index; Class.: classification; m: metacentric; sm: sub-metacentric; t: telocentric.

**Figure 3.** Karyotype of *Ensis siliqua* ($2n = 38$) with nine telocentric chromosome pairs (# 8, 9, 12, 13, 14, 15, 17, 18 and 19).**Figure 3.** Caryotype de *Ensis siliqua* ($2n = 38$), dont neuf paires de chromosomes télacentriques (n° 8, 9, 12, 13, 14, 15, 17, 18 & 19).

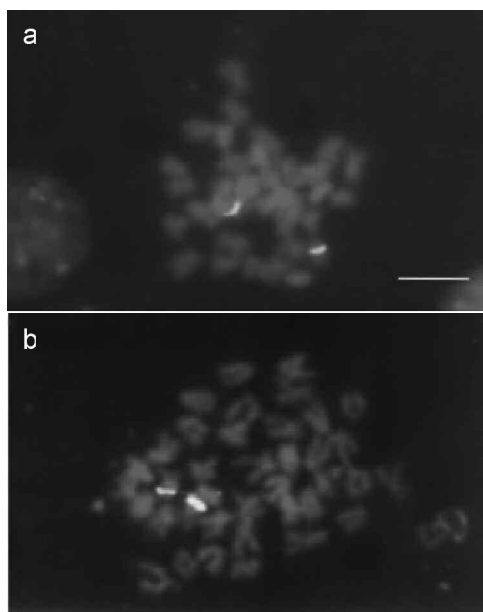


Figure 4. FISH with an 18S-5.8S-28S ribosomal probe in (a) *Ensis arcuatus* and (b) *Ensis siliqua*. Bar = 10 μ m.

Figure 4. FISH (hybridation *in situ* par fluorescence) à l'aide d'une sonde ribosomale 18S-5.8S-28S chez (a) *Ensis arcuatus* et (b) *Ensis siliqua*. Echelle = 10 μ m.

diploid chromosome number $2n = 38$, which is the most frequent in most clam species analyzed until now. Their karyotypes are very different and allow us to clearly identify one species from another and to suggest the occurrence of chromosome rearrangements during the divergence of these species. Karyotypes display a high number of telocentric chromosomes, while the rest of Veneroidea species studied show more biarmed than telocentric chromosomes (Nakamura, 1985; Thiriot-Quievreux, 1994 & 2002; Wang & Guo, 2007). In the case of superfamily Solenidae (which includes genus *Ensis* and *Solen*) cytogenetic studies are very scarce; only information about the karyotypes of *Solen constrictus*, with 18 biarmed chromosomes and one subtelocentric pair (Wang et al., 1998), *S. grandis* with 13 metacentric, three submetacentric, one subtelocentric and two telocentric pairs (Zhenxing et al., 2003), and *S. marginatus* with 11 metacentric-submetacentric, six subtelocentric and two telocentric chromosome pairs (Fernández-Tajes et al., 2003) are available. As the number of razor clam species studies is still small, it is very difficult to observe clear trends in karyotype evolution in these species. But, according to Surget-Groba et al. (2001), who pointed out that karyotypes with a high number of metacentric or submetacentric chromosomes are considered as more apomorphic than those with a high number of telocentrics, we could suppose that family Pharidae is more plesiomorphic

than the rest of venerids studied until now. This is supported by the existence of only one ribosomal locus, as pointed out by Amemiya & Gold (1990). However, the 18S-5.8S-28S locus in *E. arcuatus* and *E. siliqua* (and *S. marginatus*, too) maps at subcentromeric and subtelomeric region, respectively. This condition is unusual in other bivalve species studied, as these clusters are usually located at telomeric, and more infrequently, at subtelomeric regions (Insua et al., 1998, 1999 & 2001; Insua & Méndez, 1998; Torreiro et al., 1999; Zhang et al., 1999; González-Tizón et al., 2000; Vitturi et al., 2000; Xu et al., 2001; Martínez et al., 2002; Cross et al., 2003), with the exception of *S. marginatus* (Fernández-Tajes et al., 2003), which shows these clusters at subcentromeric and subtelomeric locations, *Hinnites distortus*, with centromeric or pericentromeric signals (López-Piñón et al., 2005), and *Mercenaria mercenaria* (Wang & Guo, 2007), with one signal near the centromere and other at the telomere.

In contrast, the existence of one locus for major rRNA genes in both *Ensis* species resulted to be, *a priori*, considered as more plesiomorphic than the existence of more than one of this locus. Other species with $2n = 38$ also showed one locus for rRNA, like *Cerastoderma edule*, *Donax trunculus*, *Macoma nasuta* and *Nutallia nutalli* (Insua et al., 1999; González-Tizón et al., 2000; Martínez et al., 2002), while *S. marginatus* (Fernández-Tajes et al., 2003) and *M. mercenaria* (Wang & Guo, 2007) showed two ribosomal loci. To explain this variation in the number of major ribosomal loci, Wang & Guo (2007) related the presence of two loci with the hypothesis of genome duplication during the evolution of bivalves, so that the existence of only one locus per genome would be consequence of the loss of one of the rRNA locus through chromosome rearrangements. In bivalves, the number of chromosome pairs carrying rRNA genes varies from one to four (Thiriot-Quievreux, 2002), so it suggests that rearrangements involving loss and gains of rRNA loci are common during bivalve evolution. Maybe, in razor clams, chromosome rearrangements could originate the loss of one of the clusters or, alternatively, the fusion of both clusters leading to one. But, instead of all these findings, more cytogenetic studies on bivalve species need to be performed in order to accurately investigate chromosome evolution.

In *E. siliqua*, the intensity of FISH signals was different. This fact is frequently observed between homologous chromosomes or, even, between two chromatids of the same chromosome (Xu et al., 2001; Wang & Guo, 2004), and it is often explained by random and local differences in chromosome denaturing, FISH conditions or as consequence of the metaphase chromosome condensation. So, in *M. californianus* and *M. trossulus* mussels (González-Tizón et al., 2000) and in *M. mercenaria* clam (Wang & Guo, 2007) some of the ribosomal loci appear

weakly labeled after FISH. These results are not surprising because rRNA genes consist of highly repeated sequences arranged in tandem, and then the differences observed in fluorescence intensity can be due to variation in copy number of the target loci (Ito et al., 2000). In eukaryotes, the existence of intra- and interindividual variability for the rDNA loci has been described for different species (Nardi et al., 1978; Mukai et al., 1991; Garrido-Ramos et al., 1995; Shishido et al., 2000).

In conclusion, this work describes for the first time the cytogenetics of *Ensis arcuatus* and *E. siliqua* and the results contribute to the number of bivalve species analyzed today.

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