Origin and evolution of *Mytilus* mussel satellite DNAs

A. Martínez-Lage*, F. Rodríguez-Fariña, A. González-Tizón, and J. Méndez

Departamento de Biología Celular y Molecular, Facultad de Ciencias, Universidade da Coruña, A Zapateira s/n, 15071 La Coruña, Spain

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Abstract

A phylogenetic reconstruction based on the amplification of 3 satellite DNAs (stDNAs) was carried out in 1 crustacean species and 15 bivalve species of the subclass Pteriomorphia (10, subfamily Mytilinae; 1, subfamily Litophaginae; 1, subfamily Modiolinae, all belonging to family Mytilidae; 1, family Arcidae; and 2, family Pectinidae). The sequences obtained showed motifs with high similarity to those of A and B boxes of tRNA promoter regions. Dot-blot hybridizations revealed that the 3 stDNAs are present mainly in high copy numbers for each species of the genus *Mytilus*, whereas for the other species they appear in low copy numbers. Maximum-parsimony trees evidenced a tendency to group *Mytilus* clones together, and species containing these sequences as a single copy were distributed among the different mytilids. Finally, the possible origin and evolution of these stDNAs is discussed.

Keywords: bivalves, Pteriomorphia, satellite DNA, phylogeny, dot-blot.

Introduction

Satellite DNA sequences are highly tandem repeated monomers mainly located at the centromeres or telomeres, or, less frequently, in interstitial chromosome regions. These repeats show high variability in sequence, frequency, and chromosomal distribution, even among closely related species (Miklos 1985; Charlesworth et al. 1994).

Usually, satellite DNAs (stDNAs) show rapid evolution among species, so that in short periods of time new families of satellite DNAs can arise or disappear (Miklos 1985). There are species-specific stDNAs, as, for example, in primates (Fanning et al. 1989) or in orthoptera (Bachmann et al. 1994), and sex-specific stDNAs, as in those from bats (Van den Busche et al. 1993) or horses (Wijers et al. 1993), and both types have a relatively recent evolutionary origin (Singer 1982). However, other stDNAs show a high degree of sequence conservation, and they are present in all the members of the same family, as observed in Cebidae (Fanning et al. 1993) or in Cetacea (Arnason 1990). These satellite sequences are widely spread across distantly related species, as, for example, the alpha-centromeric satellites of primates (Willard 1991; Fanning et al. 1993).

To explain the evolution of stDNA, Salser et al. (1976) proposed that related species share a “library” of conserved satellite sequences, some of which could be amplified, and then a larger stDNA is generated for a particular species. This model is supported by Meštrovi et al. (1998), who analyzed 4 congeneric species of

Coleoptera, each containing a different single stDNA. They proposed that these differences occur as a result of a sudden amplification of a library of conserved stDNA, its spread by a mechanism of unequal crossing over, and its fixation within some individuals in a population.

On the other hand, Nijman and Lenstra (2001) proposed a model of "the life history of satellite DNAs", which postulates that homogeneity of interacting repeating units is "both cause and consequence of the rapid turnover of stDNA". In the first phase, the amplification of homogeneous repeating units would occur, and, in the second phase, mutational events would give rise to variants that amplify independently. Later, recombination would prevent homogenization due to the spreading of 1 of the variants, and the satellite would eventually be outcompeted by another, more homogeneous tandem repeat sequence.

Studies on the evolution of stDNA in molluscs are scarce. Only Muchmore et al. (1998) in 5 species of Eastern Pacific abalone (genus *Haliotis*) and Martínez-Lage et al. (2002) in 4 *Mytilus* species carried out evolutionary studies on stDNAs. In the analysis performed by Martínez-Lage et al. (2002), 3 different types of stDNAs in *M. edulis*, *M. galloprovincialis*, *M. trossulus*, and *M. californianus* were used. The sequencing results revealed that *M. californianus* is the most divergent species with respect to the other 3. Chromosome in situ hybridization showed a different organization and distribution of the 3 satellites in each species, and the genome proportion of each type of stDNA also varied.

The central aim of the present work is to perform a molecular analysis of the 3 stDNAs used in our previous work (Martínez-Lage et al. 2002) to investigate the evolution of these sequences in the subfamily Mytilinae and analyze its possible origin and differentiation; as well, we discuss the model of "the life history of satellite DNAs" proposed by Nijman and Lenstra (2001). Specific primers were used to amplify genomic DNA from 15 bivalve and 1 crustacean species. The sequences obtained showed high similarity (with the exception of satellite 1 of *M. californianus*), although dot-blot analysis revealed that only the *Mytilus* species has a high copy number, and the rest of the species have a low copy number.

Material and methods

PCR amplifications and sequencing

The specimens analyzed were collected from the localities shown in Table 1. Clones of *M. edulis*, *M. galloprovincialis*, *M. trossulus*, and *M. californianus* were previously described by Martínez-Lage et al. (2002). DNA was obtained from adductor muscles or mantles following the protocol described in Rice and Bird (1990).

PCR amplifications from template genomic DNA were performed in a final volume of 25µL (1ng/µL) using he following primers at 0.2 mmol/L an d1Uof *Taq* DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany): S1F, 5′-TTCCATATCAACCACACATT-3′, and S1B, 5′AATACGTAAATGGGCAACTT-3′, for type 1 stDNA; S2F, 5′-ACCAAAACTCCCAAAATCAA-3′, and S2B, 5′-CCCAACAGT-TTAGGAATTAG-3′, for type 2 stDNA; and S3F, 5′-GTCACCCTACCGCTACTTTG-3′, and S3B, 5′-CATCCACCTTCTTTCGTTCA-3′ , for type 3 stDNA. The PCR amplification profile for type 1 stDNA consisted of 1 initial denaturation cycle of 3 min at 94 °C, followed by 35 amplification cycles of 1 min at 94 °C for denaturation, 30 s at 45 °C (55 and 58 °C for types 2 and 3 stDNA, respectively) for annealing, and 45 s at 72 °C for extension. A final extension cycle was performed at 72 °C for 5 min. The fragments obtained by PCR were ligated into the plasmid pGEM-T Easy Vector System (Promega Corporation, Madison, Wis.) and automatically sequenced using an ALFexpress instrument (Amersham Pharmacia Biotech, Uppsala, Sweden).

Table 1. Taxa used in the molecular analysis of 3 satellite DNAs (stDNA)

		EMBL–GenBank–DDBJ accession number*		
Taxon	**Collection site**	**Type 1 stDNA**	**Type 2 stDNA**	**Type 3 stDNA**
Class Bivalvia				
Subclass Pteriomorphia				
Family Arcidae				
Arca noae	Benicarló, Valencia, Spain	AJ549340	AJ549257	AJ549276
Family Mytilidae				
Subfamily Mytilinae				
Mytilus edulis	Yerseke, Holland	AJ420289-AJ420291	AJ420303-AJ420305	AJ420758-AJ420759
Mytilus chilensis	Puerto Aguirre, Chile	AJ549331-AJ54933	AJ549251-AJ549253	AJ549267-AJ549269
Mytilus galloprovincialis	Balcobo, La Coruña, Spain	AJ420292-AJ420296	AJ420306-AJ420308	AJ420760-AJ420762
Mytilus trossulus	Esquimalt Lagoon, Vancouver Island, B.C., Canada	AJ420297-AJ42099	AJ420309-AJ420311	AJ420763-AJ420765
Mytilus californianus	Point no Point, Vancouver Island, B.C., Canada	AJ420300-AJ420302	AJ420312	AJ420766-AJ420768
Mytilus coruscus	Otsuchi Bay, Japan	AJ549334-AJ549338	AJ549254-AJ549256	AJ549270-AJ549271
Perna canaliculus	Golden Bay, New Zealand	AJ549347	AJ549263	AJ549275
Aulacomya ater	Callao, Peru	AJ549345	AJ549260-AJ549261	AJ549274
Choromytilus chorus	Concepción, Chile	AJ549346	AJ549262	AJ549273
Septifer virgatus	Otsuchi Bay, Japan	AJ549342-AJ549344	AJ549259	AJ549277
Subfamily Lithophaginae				
Lithophaga lithophaga	Benicarló, Valencia, Spain	AJ549341	AJ549258	AJ549278
Subfamily Modiolinae				
Geukensia demissa	St. Mary's River, Md., USA	AJ549339	N.A.	AJ549272
Family Pectinidae				
Pecten maximus	O Grove, Pontevedra, Spain	N.A.	AJ549264-AJ549265	AJ549281-AJ549282
Mimachlamys varia	Málaga Bay, Spain	N.A.	N.A.	AJ549279-AJ549280
Class Crustacea				
Pollicipes cornucopia	Cedeira, La Coruña, Spain	N.A.	AJ549266	N.A.

*EMBL, European Molecular Biology Laboratory; DDBJ, DNA Data Bank of Japan; N.A., no DNA amplification with the corresponding probe.

Sequence analysis and comparison

Consensus sequencing was performed by using the internal repeats of satellite DNA sequences and the computer program CLUSTAL X (Thompson et al. 1997). Maximum-parsimony trees were constructed by close neighbor interchange search. Resolution of internal nodes was performed using 500 bootstrap iterations with random replacement (Felsenstein 1985). Phylogenetic and molecular evolutionary analyses were conducted using version 2.1 MEGA software (Kumar et al. 2001). Motifs of the internal promoters of tRNA (A and B boxes) were also analyzed using CLUSTAL X and MEGA software.

Dot-blot hybridization

Mussel populations used to estimate the copy number of the target DNA sequences in diploid genomes were *M. edulis* from Yerseke (Holland) and Prince Edward Island; *M. galloprovincialis* from Ria de Arousa, Balcobo, and Ebro Delta (all on Spanish coasts); *M. trossulus* from Öland Island (Sweden), Esquimalt Lagoon (Vancouver Island, B.C.), and Bedford Basin (Newfoundland and Labrador); *M. chilensis* from Puerto Aguirre (Chile); *M. coruscus* from Otsuchi Bay (Japan), and *M. californianus* from Point No Point (Vancouver Island, B.C.).

Solutions containing denatured genomic DNA (200, 100, 50, and 25 ng, except for Esquimalt Lagoon mussels that were 64, 32, 16, and 8 ng) and denatured *M. galloprovincialis* repetitive DNA fragments (0.8, 0.4, 0.2, and 0 ng) were vacuum-blotted onto nylon membranes in a Bio-Rad dot-blotting apparatus. Astringency washes were performed in 0.2× SSC (1× SSC: 0.15 mol NaCl/L plus 0.015 mol sodium citrate/L) 1% SDS at 50 °C. Hybridization was detected using the Dig Luminescent Detection kit for nucleic acids (Roche Molecular Biochemicals) following the manufacturer's instructions. Hybridization signals were quantified as in Martínez-Lage et al. (2003). The copy numbers of *Mytilu*s spp. repetitive sequences were calculated from a linear plot made from dilutions of M. edulis repetitive DNA fragments. The haploid DNA contents of *M. edulis* and *M. galloprovincialis* were 1.71 and 1.92 pg, respectively (Rodríguez-Juiz et al. 1996), 1.90 pg for *M. coruscus* (Ieyama et al. 1994), and 1.51 and 1.61 pg for M. trossulus and *M. californianus*, respectively (González-Tizón et al. 2000). For *M. chilensis* the haploid DNA content of *M. edulis* was used, because many investigators consider that *M. chilensis* is a subspecies of *M. edulis*.

The hybridization control for low copy number was a digoxigenin-labelled probe obtained from a PLII fragment (Heath et al. 1995). This fragment is ~425 bp in length and has a copy number ranging from 4 to 13, depending on the mussel species (Heath and Hilbish 1998; Rodríguez-Fariña 2001). To confirm that a similar DNA concentration was used in each dot-blot, a final hybridization was carried out with an rDNA probe (18S-5.8S-28S) containing ~750 copies per haploid genome.

Results

DNA amplifications with the primers previously designed by Martínez-Lage et al. (2002) yielded fragments in the ranges 170–175 bp for type 1 stDNA, 159–162 bp for type 2, and 88–89 bp to 164–167 bp for type 3. Table 1 shows the taxa used in this study. One clone from each individual was sequenced, although in the non-*Mytilus* species at least 3 clones were sequenced from each individual.

In addition, DNA amplifications were carried out with these primers in other bivalve species (data not shown): *Ostrea edulis* and *Crassostrea angulate* (family Ostreidae), *Chlamys opercularis* (family Pectinidae), *Pinna pectinata* (family Pinnidae), *Cerastoderma edule* (family Cardiidae), *Donax trunculus* (family Donacidae), *Dosinia lupinus*, *Tapes decussatus*, *Venerupis rhomboides*, and *Venerupis pullastra* (family Veneridae), *Ensis arcuatus* and *Ensis silique* (family Pharidae), and *Solen marginatus* (family

Solenidae). However, amplification fragments were not obtained in all of them. DNA amplifications from *Drosophila melanogaster* and humans were used as negative controls.

Alignments of the nucleotide sequences from type 1 stDNA (Fig. 1*a*) reveal similarity values above 86.70%, with the exception of *M. californianus* which shows about 37.00%. Comparison between *M. coruscus* (clone pCOB 105) and Aulacomya ater (pAUL 101), and between Arca noae (pARC 101) and *Lithophaga lithophaga* (pLIT 101) reveals that these species are highly similar. For type 2 stDNA (Fig. 1*b*) *A. ater* (pAUL 202) and *Perna canaliculus* (pPER 201) display the highest sequence similarity, and the other species exhibit values above 84.50%, with the exception of *M. californianus* (values close to 75.00%).

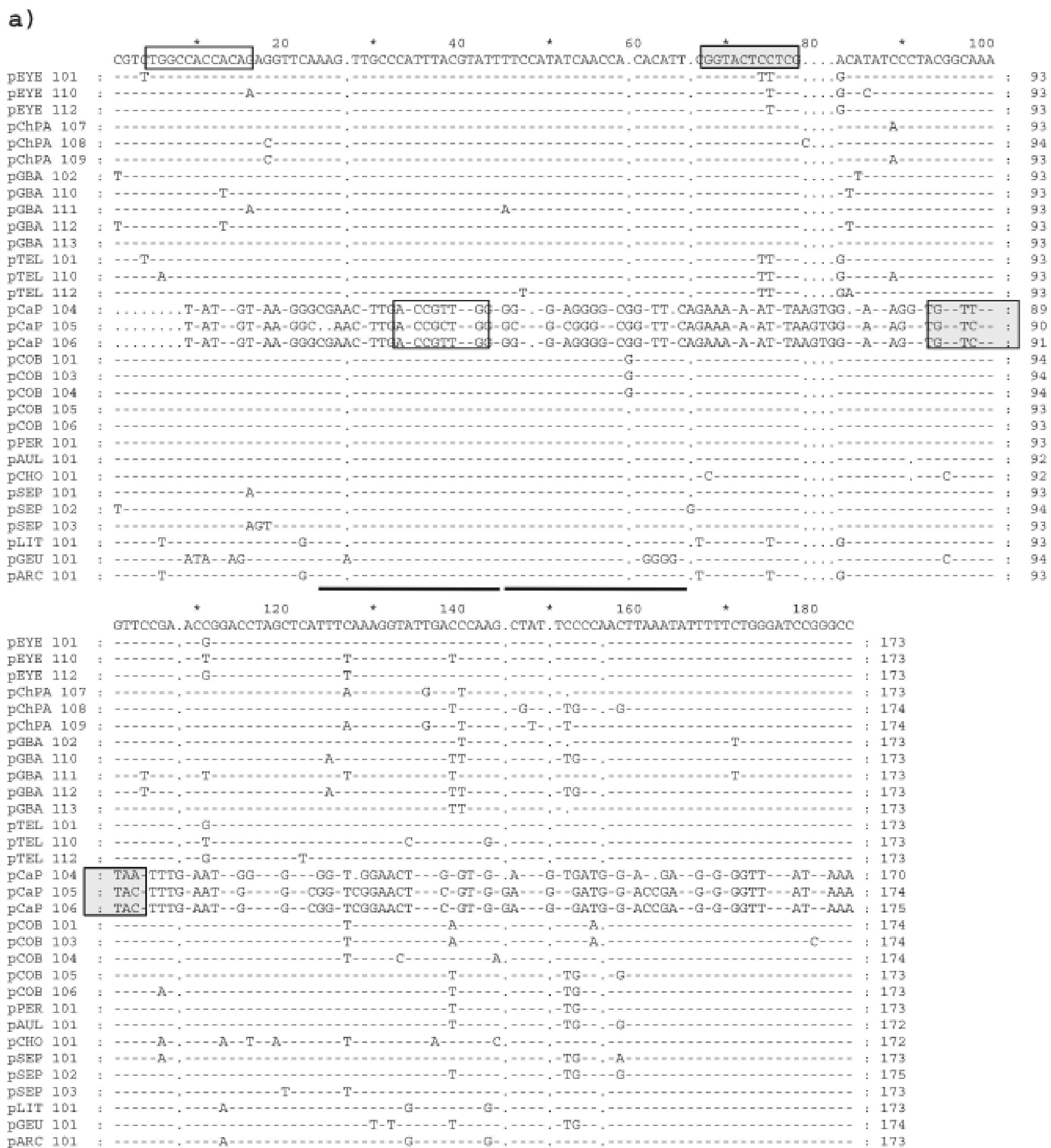


Figure 1. Nucleotide sequences of a) DNA type 1, b) DNA type 2, and c) DNA type 3. Monomers derived from the same cloned fragments are indicated as suffixes: EYE as *Mytilus edulis*, ChPA as *M. chilensis* ,GBAas *M. galloprovincialis*, TEL as *M. trossulus*, CaP as *M. californianus*, COB as *M. coruscus*, PER as *Perna canaliculus*, AUL as *Aulacomya ater* ,CHOas *Choromytilus chorus* , SEP as *Septifer virgatus* ,LITas *Lithophaga lithophaga* , GEU as *Geukensia demissa*, ARC as *Arca noae*, POL as *Pollicipes cornucopia*, PEC as *Pecten maximus*, and CHL as *M. varia*. Only nucleotides divergent from the top sequences are indicated in particular monomers, and gaps are shown with dots.

White and grey boxes indicate similarity with A box (5 ′-TRGCNNAGY*GG-3′) and B box (5′-GGTTCGANTCC-3′), respectively, internal promoters of tRNA (Geiduschek and Tocchini-Valentini 1988). Primer binding sites are underlined.

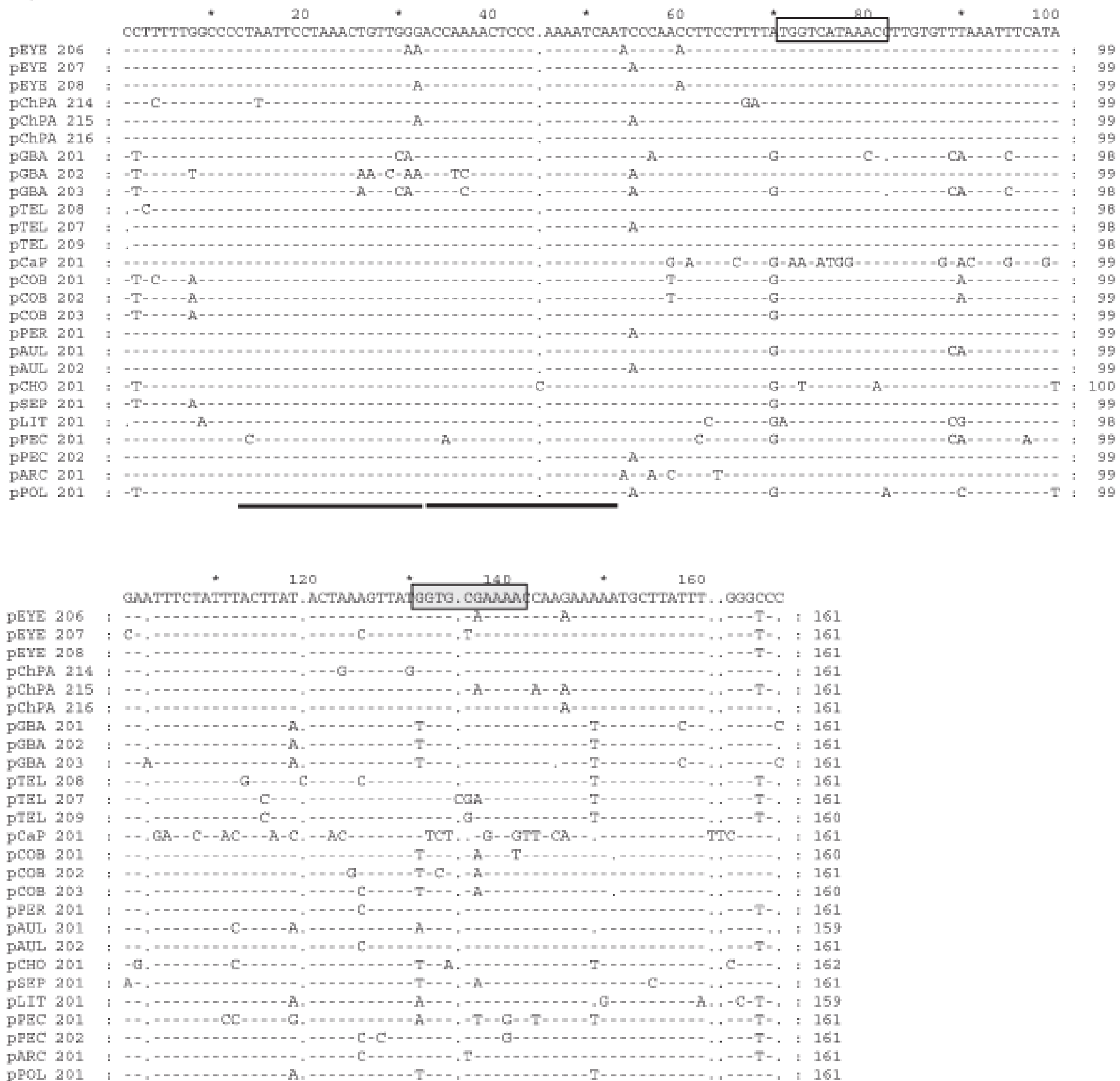


Figure 1*c* shows that *Septifer virgatus* (pSEP 301) and *L. litophaga* (pLIT301) are the most divergent among the species, displaying a similarity value of 60.00%. The rest of the species show similarity values above 84.50%. *M. californianus* is less divergent for satellite type 3 than for satellite types 1 and 2.

The sequences obtained have been aligned, showing the motifs that display a certain similarity with those of A box and B box of promoter regions of tRNAs. In *M. californianus*, this motif is different from that in the rest of the species (Fig. 1 *a*). Similarity values are about 63.64% in A box and B box of type 1 stDNA, increasing to 72.73% for A box in *M. californianus* . In the case of type 2 stDNA, the similarity value for A box is 45.46% and for B box is 72.73%. In type 3 stDNA, similarity values are 81.82% and 54.55% for A box and B box, respectively. Finally, A and B boxes in *S. virgatus* and *L. litophaga* do not show similarity with any of the other species.

c)

Results from dot-blot hybridizations (Fig. 2) reveal that these stDNA sequences are present in high copy numbers for each species of the genus *Mytilus* (*M. edulis*, *M. galloprovincialis*, M. trossulus, *M. chilensis*, *M. coruscus*, and *M. californianus*), whereas for the other species they are single copy sequences because fewer than 10 copies are present. Table 2 shows the copy number and proportion of each satellite DNA in each population analyzed. For copy number, a notable difference exists between *M. californianus* and the other species used. Another remarkable finding is the high copy number and proportion of type 3 stDNA in *M. trossulus* species.

The pairwise comparison of Kimura 2-parameter distance and transition/transversion (ts/tv) (data not shown; provided upon request) ratios for type 1 stDNA showed that distance values tend to be lower than 0.10; for *Geukensia demissa*, values ranged from 0.09 to 0.15, and *M. californianus* clones showed the highest distance values (1.22–2.17). On the other hand, transitions are more abundant than transversions, as evidenced by ts/tv ratios greater than 1.0. For type 2 stDNA, distance values ranged from 0.01 to 0.36, again *M. californianus* showing the highest values. Like type 1 stDNA, type 2 shows ts/tv values above 1.0. For type 3 stDNA, distance values ranged from 0.01 to 0.49, those corresponding to *S. virgatus* and *L. litophaga* being the highest. With a few exceptions, ts/tv ratios were less than 1.0.

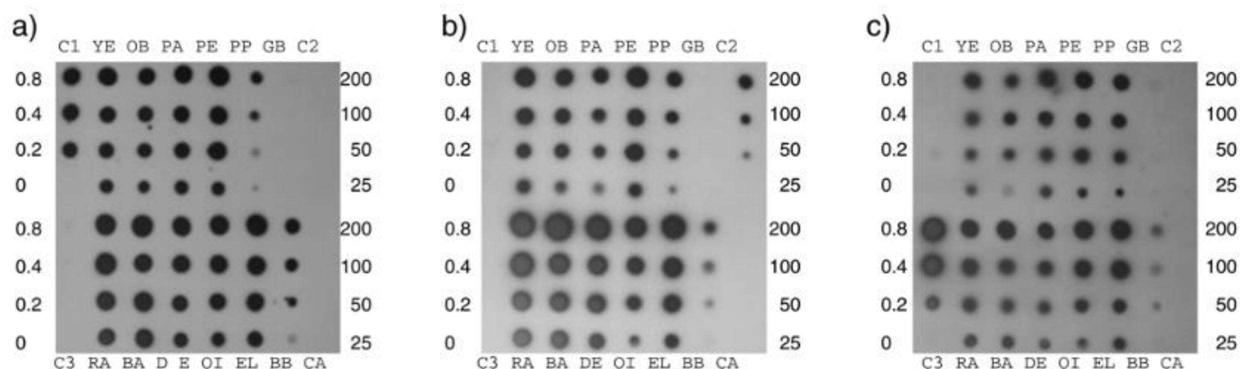


Figure 2. Dot-blot hybridizations from total genomic DNAs: *Mytilus edulis* from Yerseke (YE) and Prince Edward Island (PE); *M. coruscus* from Otsuchi Bay (OB); *M. chilensis* from Puerto Aguirre (PA); *M. californianus* from Point No Point (PP); *Perna canaliculus* from Golden Bay (GB); *M. galloprovincialis* from Ria de Arousa (RA), Balcobo (BA), and Delta del Ebro (DE); *M. trossulus* from Öland Island (OI), Esquimalt Lagoon (EL), and Bedford Basin (BB); and *Aulacomya ater* from El Callao (CA). C1, C2, and C3 correspond to *M. edulis* satellite DNA fragments, types 1, 2, and 3, respectively. Hybridizations with (a) type 1 stDNA, (b) type 2 stDNA, and (c) type 3 stDNA. Amounts of blotted DNA are 200, 100, 50, and 25 ng for genomic DNAs (except for Esquimalt Lagoon, which are 64, 32, 16, and 8 ng); for repeated DNA controls, 0.8, 0.4, 0.2, and 0 ng.

As shown in the maximum-parsimony trees (Fig. 3), clones containing stDNA sequences (genus *Mytilus*) tend to group together, whereas clones containing these sequences as single copy are distributed among the different mytilids. The topology of the tree obtained for type 1 stDNA (Fig. 2 *a*) shows a cluster containing all the *M. californianus* clones and another cluster with *M. coruscus* clones. *M. chilensis* and *M. galloprovincialis* cluster together (with the exception of 1 clone for each species), as do *M. edulis* and *M. trossulus* . The 3 clones of *S. virgatus* are dispersed among the other species. The topology for type 2 stDNA (Fig. 2b) reveals that genus *Mytilus* clones do not group together, with the exception of *M. coruscus*. For type 3 stDNA only *M. coruscus* clones group together in the same cluster with *A. ater* and *A. noae* ; the other mytilid clones group separately.

Table 2. Copy number and proportion for the different satellite DNAs (stDNA) in 11 *Mytilus* mussel populations.

	Copy number and % genomic content					
	Type 1 stDNA		Type 2 stDNA		Type 3 stDNA	
M. edulis						
Yerseke	45000	0.46%	140000	1.31%	1100	0.01%
Prince Edward Island	53000	0.54%	120000	1.11%	1400	0.01%
M. galloprovincialis						
Ría de Arousa	69000	0.63%	150000	1.27%	4400	0.04%
Balcobo	79000	0.73%	200000	1.66%	3500	0.03%
Delta del Ebro	71000	0.65%	180000	1.55%	2800	0.03%
M. trossulus						
Oland Island	60000	0.70%	110000	1.13%	7400	0.08%
Esquimalt Lagoon	70000	0.79%	140000	1.51%	11000	0.12%
Bedford Bassin	60000	0.71%	130000	1.36%	8000	0.09%
M. chilensis						
Puerto Aguirre	37000	0.38%	110000	1.04%	1100	0.01%
M. coruscus						
Otsuchi Bay	35000	0.37%	110000	1.08%	1600	0.02%
M. californianus						
Point No Point	8500	0.10%	80000	0.85%	10000	0.11%

Discussion

The previous characterization of 3 satellite DNAs in the mussel species *M. edulis*, *M. galloprovincialis*, *M. trossulus*, and *M. californianus* (Martínez-Lage et al. 2002) revealed that monomeric lengths were 171 bp for type 1 satellite DNA, 161 bp for type 2 satellite DNA, and 167 bp for type 3. The determination of the genomic contents of these repetitive sequences showed that type 2 satellite DNA was the most abundant of the 4 mussel species, and type 3 showed the lowest content. Fluorescent in situ hybridization revealed a random distribution of these repetitive sequences into several small clusters dispersed along the chromosomes.

In the present study, these repetitive DNA sequences were analyzed in other *Mytilus* species and in bivalve species closely related to the genus *Mytilus* (Table 1). The results reveal that monomeric lengths and sequence similarities are very like those reported for the 4 *Mytilus* mussels previously analyzed (Martínez-Lage et al. 2002) and that single-point mutations are the major contributors to divergence among them. However, there is interspecific variability in copy number, so that only the species belonging to the genus *Mytilus* present these sequences as satellite DNAs (high copy number), whereas in the rest of the species they appear as single copy or as low copy number. DNA from *D. melanogaster* and humans was used as a negative control because the DNA from the crustacean *Pollicipes cornucopia* (initially selected as a negative control) was amplified with type 2 stDNA. On the other hand, the analyses of different populations of *M. edulis*, *M. galloprovincialis*, and *M. trossulus* showed very slight differences in copy number.

Repetitive sequences can be classified according to the genetic mechanism by which they originate. DNA duplications create tandemly repeated sequences, whereas retrotransposition events generate dispersed elements (Ohshima et al. 1993). As observed in the sequence alignments (Fig. 1), these repetitive sequences show a region similar to those in A and B tRNA boxes, which initially suggests that they could be tRNA-derived pseudogenes. As Oshima et al. (1993) and Ohshima and Okada (1994) suggested, these types of sequence are usually derived by retroposition. Under the "selfish" DNA hypothesis, repetitive sequences would accumulate in heterochromatin because these regions do not contain genes, and, subsequently, they are less likely to be deleterious, although accumulation of highly repetitive sequences is expected to occur only in regions where there is very low recombination and weak selective constraints on array length (Charlesworth et al. 1986, 1994; Stephan 1989). Under the same hypothesis, this process would occur by transposition and subsequent increase in their copy number. However, the possibility of horizontal transfer of satellite repeats among species is highly improbable because of the distribution of these sequences among taxonomically related species and the absence of data confirming the occurrence of the same satellite DNA in phylogenetically unrelated groups (Meštrovi et al. 1998). Our results agree with the library hypothesis proposed by Nijman and Lenstra (2001). These authors suggested that related species share a library of conserved satellite sequences, some of which could be amplified, creating a larger satellite DNA in a particular species. So, these sequences would have spread to constitute bigger satellite DNAs in the genus *Mytilus* species, but in the rest of the "non-*Mytilus* " species would have remained as a single copy or low copy number. Probably, the expansion of these 3 satellite DNAs could have occurred in the recent Jurassic, 150 million years ago, when the genus *Mytilus* emerged (Cox et al. 1969).

The high sequence similarity displayed among the 3 types of sequence (except for type 1 stDNA in *M. californianus*) suggests that types 1, 2, and 3 repetitive sequences are in the initial phase of the model proposed by Nijman and Lenstra (2001). This model is known as "life history of satellites" and it postulates the existence of 3 phases in the evolution of satellite DNAs. During the initial phase, interactions of homogeneous repeating units cause rapid expansions and contractions, leading to saltatory fluctuations in the copy number. Perhaps, in *M. californianus*, type 1 stDNA would be in the second phase of this model, when mutations and recombination are acting to lead to divergence of sequence variants. Then, taking into account transition/transversion values, as in Kimura's 2-parameter model, Li (1997) proposed that when a sequence is recent (≤ 50 m.y. old) ts/tv ratios are above 1.0. When the evolutionary time is approximately 50

million years ago, the ts/tv ratios are 1.0 or close to 1.0, and when it is more than 200 million years these ratios are 0.5. According to Li (1997), type 3 stDNA in *M. californianus* would be older than types 1 and 2, but, according to the results of Salser et al. (2001), the oldest would be type 1 stDNA. There are 2 possibilities to explain the origin of these 3 types of satellite DNAs: (i) that each occurred at different evolutionary times, or ((ii) that the 3originated at the same time but by different evolutionary mechanisms (gene conversion, recombination, mutation), these being faster or more active in type 1 stDNA than in types 2 and 3. The parsimony trees obtained in this analysis (Fig. 3) show the divergence among clones of different species, and also the tendency of clones of the same *Mytilus* species to group in a single cluster. This does not always occur, perhaps because stDNA clusters are dispersed throughout the genomes of these species.

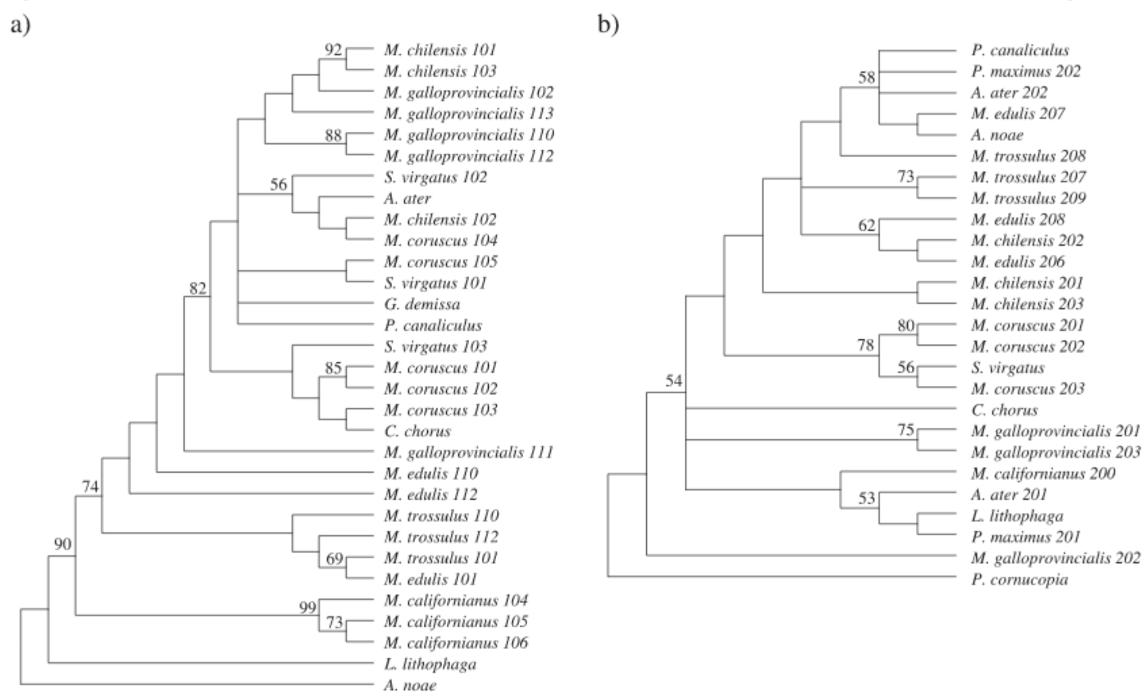
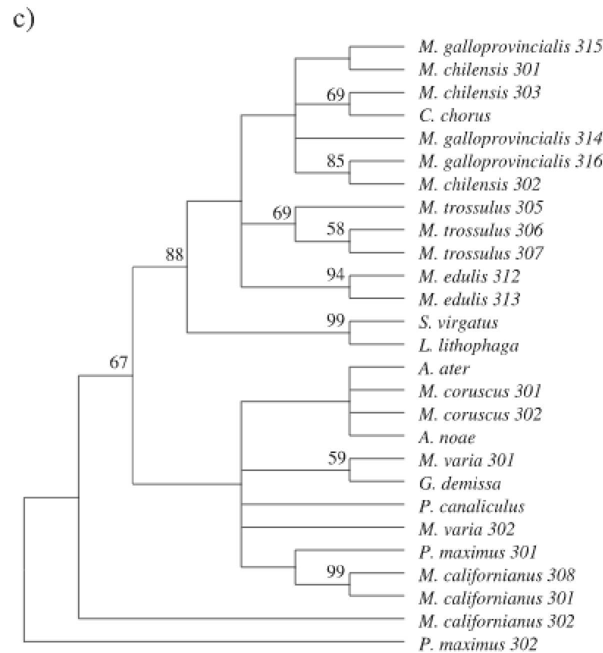


Figure 3. Maximum-parsimony bootstrap (500 replicates) consensus trees for (a) DNA type 1, (b) DNAtype2,and (c) DNA type 3. Bootstrap values over 50% are shown above branches. The numbers at the end of the branches refer to the number of the clone analyzed.

Different studies were conducted to analyze phylogenetic relations among members of the Mytilinae subfamily using nuclear ribosomal DNA sequences (Kenchington et al. 1995; Steiner and Müller 1996; Winnepenninckx et al. 1996; Adamkewicz et al. 1997; Distel 2000) or mitochondrial DNA (Edwards and Skibinski 1987; Geller et al. 1993; Hilbish et al. 2000). None of these analyses grouped *M. edulis* clones and *M. galloprovincialis* clones separately, however. Our analysis with stDNAs shows both species in different clusters (Fig. 3). In the case of *M. trossulus* clones, phylogenies obtained from mitochondrial DNA sequences show them grouping in well-defined clusters (Geller et al. 1993; Hilbish et al. 2000), whereas those obtained from nuclear ribosomal DNA appear in an ambiguous position (Kenchington et al. 1995; Distel 2000). In all the studies performed until now, *M. californianus* has proved to be the most divergent species (Kenchington et al. 1995; Distel 2000; Hilbish et al. 2000; Eirín-López et al. 2002; present work). Finally, allozyme analysis has shown *M. chilensis* to be a subspecies of *M. edulis* (McDonald et al. 1991), but data obtained from mitochondrial DNA (Hilbish et al. 2000), nuclear genes (Rodríguez-Fariña 2001), and satellite DNAs (present study) indicate that this species is closer to *M. galloprovincialis* than to *M. edulis*. These results support the taxonomy of Lamy (1936), who proposed that *M. chilensis* is a species apart from

M. edulis and *M. galloprovincialis* In conclusion, our results support the library hypothesis proposed by Salser et al. (1976), in that the analyzed sequences were present as a single copy/low copy number in the non-*Mytilus* species and as satellite DNAs in the *Mytilus* species. These sequences must be analyzed in other bivalve species to investigate the extension of this library.



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[*] andres@udc.es