

Chromosomal Rearrangement in Pectinidae Revealed by rRNA Loci and Implications for Bivalve Evolution

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Abstract. Karyotype and chromosomal localization of major (18–5.8–28S) and minor (5S) ribosomal RNA genes were studied in two species of Pectinidae, zhikong (*Chlamys farreri*) and bay (*Argopecten irradians irradians*) scallops, using fluorescence *in situ* hybridization (FISH). *C. farreri* had a haploid number of 19 with a karyotype of 3m + 4sm + 7sm-st + 4st + 1st-t, and *A. i. irradians* had a haploid number of 16 with a karyotype of 5st + 11t. In *C. farreri*, the major and minor rRNA genes had one locus each and were mapped to the same chromosome—Chromosome 5. In *A. i. irradians*, the major rRNA genes had two loci, located on Chromosomes 4 and 8, and the 5S rRNA gene was found at a third chromosome—Chromosome 10. Results of this and other studies indicate that karyotype of *A. i. irradians* ($n = 16$, 21 arms) is secondary and derived from an ancestral karyotype similar to that of *C. farreri* ($n = 19$, 38 arms) through considerable chromosomal loss and rearrangements. The ability to tolerate significant chromosomal loss suggests that the modal karyotype of Pectinidae and possibly other bivalves with a haploid number of 19 is likely tetraploid; *i.e.*, at least one genome duplication has occurred during the evolution of Bivalvia.

Introduction

Chromosomal changes, particularly polyploidy, have played a significant role in the evolution of plants, and most higher plants are recent polyploids (DeWit, 1980). Although polyploidy is relatively rare in animals, chromosomal changes are increasingly recognized as an important force in

animal evolution. It is hypothesized that two rounds of genomic duplication occurred during the evolution of vertebrates leading to humans (Furlong and Holland, 2002; Spring, 2002). Chromosomal rearrangements may play a role in reproductive isolation and speciation, by creating barriers to meiotic pairing and reducing the fitness of hybrids (White, 1978; King, 1993). Genic theories, on the other hand, stress the importance of accumulation of genic mutations in reproductive isolation. Recent findings of effects of chromosomal rearrangements on recombination have bridged the gap between the chromosomal and genic theories of reproductive isolation, arguing for a major role of chromosomal changes in speciation (Rieseberg, 2001; Navarro and Barton, 2003). However, the extent of chromosomal changes and their roles in speciation are poorly understood in many animal taxa, including marine bivalves. Many marine bivalves are sympatric broadcast spawners whose mechanisms of reproductive isolation are particularly interesting but largely unknown. Chromosomal studies may provide a unique perspective on the evolution of marine bivalves.

Scallops, members of family Pectinidae, are widely distributed in world oceans. Scallops are characterized by two mostly equal and round valves with a byssal notch and ctenolium on the right valve. Most scallops are free living and mobile, and inhabit the surface of subtidal bottoms. Some species may use byssal threads for temporary attachment or cement themselves permanently onto hard surfaces. The earliest representative of Pectinidae appeared in the Triassic period about 230 million years ago, but most groups did not emerge until the early Paleocene, or 65 million years ago (Waller, 1991). The family contains about 360 living species, and many of them are important for

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fishery and aquaculture production. Chromosome number and karyotype have so far been studied in 16 species of scallops (Beaumont and Gruffydd, 1974; Komaru and Wada, 1985; Insua *et al.*, 1998; Pauls and Affonso, 2000). Chromosome number and karyotype vary considerably. While most of the species have a haploid number of 19 chromosomes, some have 16 (Wada, 1978; Komaru and Wada, 1985), and one species, *Aequipecten opercularis*, has 13 (Beaumont and Gruffydd, 1974). Even among species with 19 pairs of chromosomes, variation in karyotype is apparent, and the number of telocentric chromosomes varies from zero in *Chlamys farreri* to 14 in *Pecten maximus* and *P. albicans* (Beaumont and Gruffydd, 1974; Komaru and Wada, 1985).

Clearly, significant changes in chromosome number and structure occurred during the evolution of Pectinidae. However, detailed analysis of chromosomal changes in scallops is hindered by the inability to identify individual chromosomes. Chromosome identification remains difficult in scallops as well as in most marine bivalves, primarily due to the lack of cell lines needed for preparing elongated chromosomes. Chromosome identification by traditional banding and using embryonic material is possible, but not practical. C-banding analysis has been studied in the queen scallop *Aequipecten opercularis*, but banding characteristics were not distinctive or reliable for routine chromosome identification (Insua *et al.*, 1998). Nucleolar organizer regions (NORs) have been studied in the queen scallop and the Chilean-peruvian scallop *Argopecten purpuratus* (Insua *et al.*, 1998; Gajardo *et al.*, 2002), although NOR sites are not always reliable as chromosomal landmarks (Pauls and Affonso, 2000; Wang *et al.*, 2004). Recently, fluorescence *in situ* hybridization (FISH) has been used for chromosomal identification and karyotypic analysis in marine bivalves, providing clear and unambiguous identification of some chromosomes (*e.g.*, Insua *et al.*, 2001; Xu *et al.*, 2001; Wang *et al.*, 2004). The major (18S–5.8S–28S) and minor (5S) ribosomal RNA genes have been assigned to the chromosomes of *Aequipecten opercularis* (Insua *et al.*, 1998).

Major and minor rRNA genes are two distinct families of ribosomal RNA genes in higher eukaryotes. The two gene families are relatively independent of each other and often organized into separate loci on the same or different chromosomes (Martins and Galetti, 2001; Liu *et al.*, 2002). The major rRNA genes correspond to NORs and sometimes can be visualized by silver-staining. Both gene families are present in large numbers of tandem repeats, making them ideal targets for FISH. We studied the karyotype and chromosomal localization of the major and 5S rRNA genes by FISH in Zhikong (*C. farreri*) and bay (*A. irradians irradians*) scallops, two Pectinid species with different haploid numbers. Surprisingly, the species with the lower haploid number (*A. i. irradians*, $n = 16$) had three rRNA-bearing chromosomes, while the species with the higher haploid

number (*C. farreri*, $n = 19$) had one, suggesting that the karyotype of the latter is pleisomorphic.

Materials and Methods

Chromosome preparation

The Zhikong scallops (*Chlamys farreri* Jones and Preston 1904) and bay scallops (*Argopecten irradians irradians* Lamarck 1819) used in this study were from hatcheries in Shandong, China. *C. farreri* is a native species of China and commonly found along much of that country's north and central coasts as well as in waters off Korea and Japan. *A. i. irradians* is native to the Atlantic coast of North America, and it was introduced to China in 1982 (Zhang *et al.*, 1986). *C. farreri* and *A. i. irradians* are the two most important scallop species cultured in China.

Metaphase chromosomes were prepared from early embryos, using the procedures described in Guo *et al.* (1992). Briefly, early embryos were treated with 0.005% colchicine for 15 min and 0.075 M KCl for 12 min, before being fixed in freshly prepared fixative, 1:3 (v:v) acetic acid and methanol. The fixative was changed at least twice, with a 15-min duration each time. The fixed samples were stored in the fixative at 4 °C until use. Chromosome samples were prepared by loading the fixed cell suspension onto preheated slides and air-dried. Slides were stored at 4 °C before FISH analysis.

Probe construction

Genomic DNA was extracted from adductor muscle by proteinase K digestion, according to the method described by Doyle and Doyle (1987). A fragment of the targeted ribosomal RNA locus was amplified, labeled with digoxigenin-11-dUTP (alkali-stable) by PCR incorporation. Digoxigenin-11-dUTP and all other PCR reagents were purchased from Roche Diagnostics (Indianapolis, IN). Intergenic transcribed spacers between the 18S and 5.8S rRNA genes (ITS1) were used as probes for the major rRNA genes. The primer sequences were 5'-GGTTTCTGTAGGTGAACCTGC and 5'-CTGCGTTCTTCATCGACCC. For the 5S rRNA gene, primers were designed based on the published sequence of mussel *Mytilus edulis* 5S rRNA (GenBank accession no. J01869; Fang *et al.*, 1982): 5'-GTCTACGACCATATCACGTTGAAAA and 5'-TGTC-TACAACACCCGGTATTCCC. The PCR reaction mixture (25 μ l) contained 1.5 mM of MgCl₂; 0.2 mM each of dATP, dCTP, and dGTP; 0.13 mM dTTP; 0.07 mM digoxigenin-11-dUTP (for labeling); 0.63U Taq DNA polymerase; 0.4 mg/ml BSA; 1 μ M of each primer; and 1 μ g of genomic DNA. The PCR reaction was performed using a DeltaCycler II System thermal cycler, with an initial 5-min denaturation at 95 °C; followed by 35 cycles of 1-min denaturation at 95 °C, 1-min annealing at 50 °C, and 1-min extension at 72 °C;

and a final 5-min extension at 72 °C. PCR products were evaluated on 1% (w:v) agarose gels and visualized by 1 µg/ml ethidium bromide staining and ultraviolet illumination.

Fluorescence in situ hybridization

FISH was carried out according to Guo and Allen (1997a), with slight modifications. Chromosomes were pretreated by incubating the slides in 2× SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0) for 30 min at 37 °C; dehydrated in 70%, 80%, and 95% ethanol for 2 min each; and air-dried. Chromosomes were denatured in 70% formamide in 2× SSC (pH 7.0) at 72 °C for 2 min and then dehydrated in a cold ethanol series (70%, 80%, and 95%) and air-dried. The labeled probes were diluted in hybridization solution, 65% formamide in 2× SSC, at a ratio of 1:15; denatured at 72 °C for 5 min; and placed immediately on ice. Probe mixture (15–20 µl) was applied to each denatured slide, covered with a glass coverslip, and sealed with rubber cement. For dual-hybridization of two probes on the same metaphase, two probes were denatured and mixed before application. Slides were then incubated at 37 °C in a humidity incubator overnight for hybridization. After hybridization, the coverslips were removed and the slides were washed twice in 2× SSC at 72 °C for 5 min each time, with 1× PBT (0.1 M NaH₂PO₄, 0.4% BSA, 0.1% Tween-20, pH 7.4) at room temperature for 2 min. The digoxigenin-labeled probes were detected with fluorescein-labeled

anti-digoxigenin antibody. Chromosomes were counterstained with 0.6 µg/ml of propidium iodide (PI) in anti-fade solution (Vector Laboratories) and viewed under a Nikon epifluorescence microscope. FISH signals and karyotype were captured using a 3CCD camera and analyzed using the Image-Pro Plus 3.0 software. Chromosomes were classified according to the criteria defined by Levan *et al.* (1964).

Results

Probe quality

All PCR amplifications were successful and produced specific fragments as visualized on agarose gels. Amplification with ITS1 primers in *Chlamys farreri* generated a single fragment at about 350 bp (Fig. 1A, Lane 2). The incorporation of digoxigenin-11-dUTP shifted the PCR product to about 480 bp (Fig. 1A, Lane 3), a sign of successful labeling. In *Argopecten irradians irradians*, the ITS1 fragment was slightly longer than that from *C. farreri*, or about 380 bp (Fig. 1B, Lane 2). The labeling with digoxigenin-11-dUTP shifted the fragment to about 500 bp.

PCR amplification with 5S rRNA primers generated a single fragment of about 120 bp, the expected size, in both species (Fig. 1A, B, Lane 4). Similarly, the mobility of the PCR product shifted to about 200 bp after the incorporation of digoxigenin-11-dUTP (Lane 5), indicating that the labeling was successful.

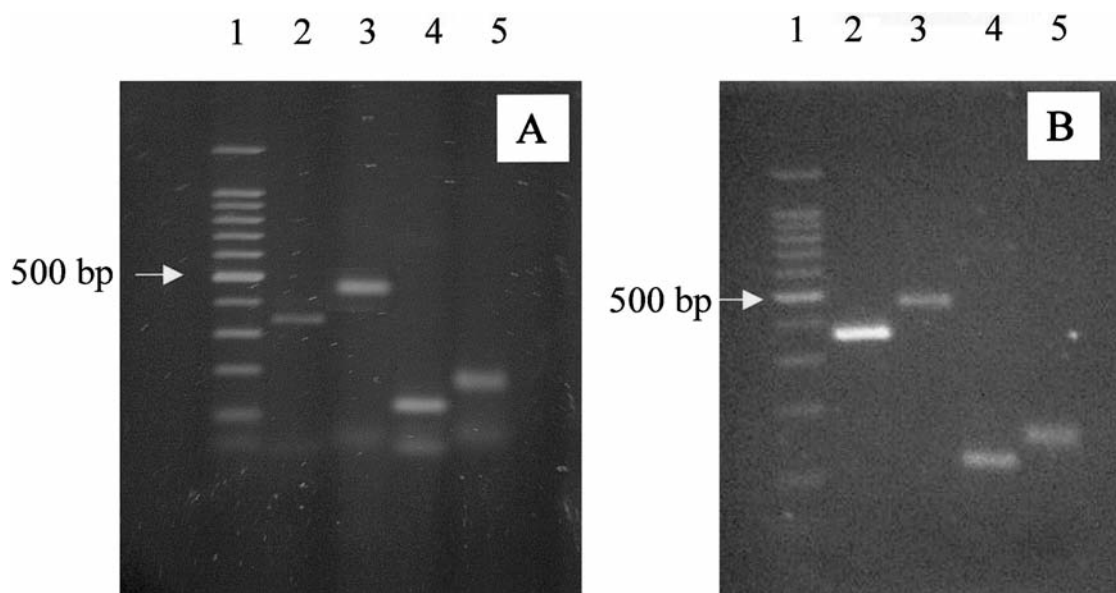


Figure 1. PCR products of intergenic transcribed spacer between 18S and 5.8S rRNA genes (ITS1) and 5S rRNA genes from *Chlamys farreri* (A) and *Argopecten irradians irradians* (B). Lane 1, 100-bp DNA ladder; Lanes 2 and 3, unlabeled and digoxigenin-labeled ITS1 products, respectively; Lanes 4 and 5, unlabeled and digoxigenin-labeled 5S rRNA gene products, respectively. Arrows indicate the location of 500-bp fragment.

Karyotype

Little variation in haploid number was observed among the metaphases screened in this study. The loss of one or two chromosomes was occasionally seen in a few metaphases that appeared to be overly spread. Analyses confirmed the haploid numbers previously reported for both species: 19 for *C. farreri* and 16 for *A. i. irradians*.

Fifteen metaphases that showed no chromosome overlapping or signs of chromosome loss were selected for karyotypic analysis. Chromosomes were measured after FISH. In addition to the difference in haploid number, the two species had strikingly different karyotypes. The karyotype of *C. farreri* consisted of the following chromosome types: three metacentric (m), four submetacentric (sm), seven submetacentric or subtelocentric (sm-st, centromeric index \pm SD overlaps two categories), four subtelocentric (st), and one subtelocentric or telocentric (st-t) (Table 1). In comparison, the karyotype of *A. i. irradians*, 5st + 11t, contained only subtelocentric and telocentric chromosomes and no metacentric or submetacentric chromosomes (Table 2). Although the pairing of some chromosomes is subjective because of similarities in length and arm ratio, the number of different types of chromosomes clearly set the two karyotypes apart.

18S-5.8S-28S rRNA genes

The major rRNA genes were easily detected with the ITS1 probes. Strong FISH signals were found on interphase

Table 1

Karyotype analysis of 15 metaphases in zhikong scallop Chlamys farreri

Chromosome no.	Relative length (mean \pm SD)	Centromeric index (mean \pm SD)	Classification ¹
1	6.79 \pm 0.15	0.31 \pm 0.03	sm
2	6.60 \pm 0.25	0.23 \pm 0.03	sm/st
3	6.46 \pm 0.15	0.29 \pm 0.04	sm
4	6.17 \pm 0.24	0.23 \pm 0.02	st
5	5.95 \pm 0.32	0.25 \pm 0.03	sm/st
6	5.78 \pm 0.25	0.31 \pm 0.03	sm
7	5.60 \pm 0.16	0.23 \pm 0.04	sm/st
8	5.36 \pm 0.39	0.28 \pm 0.05	sm/st
9	5.35 \pm 0.35	0.43 \pm 0.03	m
10	5.27 \pm 0.04	0.25 \pm 0.03	sm/st
11	5.03 \pm 0.12	0.20 \pm 0.04	st
12	4.86 \pm 0.20	0.28 \pm 0.04	sm/st
13	4.78 \pm 0.47	0.11 \pm 0.02	st/t
14	4.77 \pm 0.32	0.41 \pm 0.05	m
15	4.62 \pm 0.16	0.25 \pm 0.03	sm/st
16	4.45 \pm 0.20	0.22 \pm 0.01	st
17	4.16 \pm 0.11	0.27 \pm 0.02	sm
18	4.01 \pm 0.24	0.42 \pm 0.03	m
19	3.97 \pm 0.21	0.15 \pm 0.02	st

¹ m = metacentric, sm = submetacentric, st = subtelocentric, t = telocentric; m/sm, sm/st, and st/t are chromosomes overlapping two categories.

Table 2

Karyotype analysis of 15 metaphases in bay scallop Argopecten irradians irradians

Chromosome no.	Relative length (mean \pm SD)	Centromeric index (mean \pm SD)	Classification ¹
1	8.40 \pm 0.52	0	t
2	8.23 \pm 0.59	0.15 \pm 0.01	st
3	7.73 \pm 0.40	0	t
4	6.95 \pm 0.24	0.20 \pm 0.02	st
5	6.75 \pm 0.06	0	t
6	6.39 \pm 0.36	0.20 \pm 0.02	st
7	6.23 \pm 0.16	0	t
8	6.21 \pm 0.39	0.16 \pm 0.02	st
9	5.98 \pm 0.09	0	t
10	5.82 \pm 0.40	0	t
11	5.70 \pm 0.11	0	t
12	5.53 \pm 0.13	0	t
13	5.34 \pm 0.32	0	t
14	5.02 \pm 0.29	0.20 \pm 0.02	st
15	5.07 \pm 0.08	0	t
16	4.64 \pm 0.20	—	t

¹ st = subtelocentric; t = telocentric.

nuclei and metaphase chromosomes in both species. The number of signals per nucleus was variable, which was expected because of varying stages of the cell cycle and overlapping signals. The number of signals per metaphase was consistent. In *C. farreri*, the number of FISH signals per nucleus varied between one and two. For metaphases, FISH signals were consistently found on two (or one pair of) chromosomes (Fig. 2A). Karyotypic analysis indicated that the FISH signals were located on the telomeric region of the short arm of Chromosome 5 (Fig. 2H), a submetacentric or subtelocentric chromosome with a centric index of 0.25 ± 0.03 . In *A. i. irradians*, two to four FISH signals were observed in interphase nuclei. For metaphases, FISH signals were observed on two pairs of chromosomes (Fig. 2E). Karyotypic analysis showed that the FISH signals were located on Chromosomes 4 and 8 (Fig. 2I). Both chromosomes were subtelocentric, and the FISH signals were in telomeric regions of the short arms.

5S rRNA genes

Compared with the FISH signals generated by the ITS1 probe for major rRNA genes, those from the 5S probe were relatively weak, but strong enough for unambiguous chromosomal assignment. As with ITS1, the number of signals varied in interphase nuclei but was consistent on metaphase chromosomes. In both species, FISH signals were found on two (or one pair of) chromosomes (Fig. 2B, F). In both species, the 5S locus was at an interstitial site on the long arms, about 1/3 arm length away from the telomere and 2/3 arm length from the centromere.

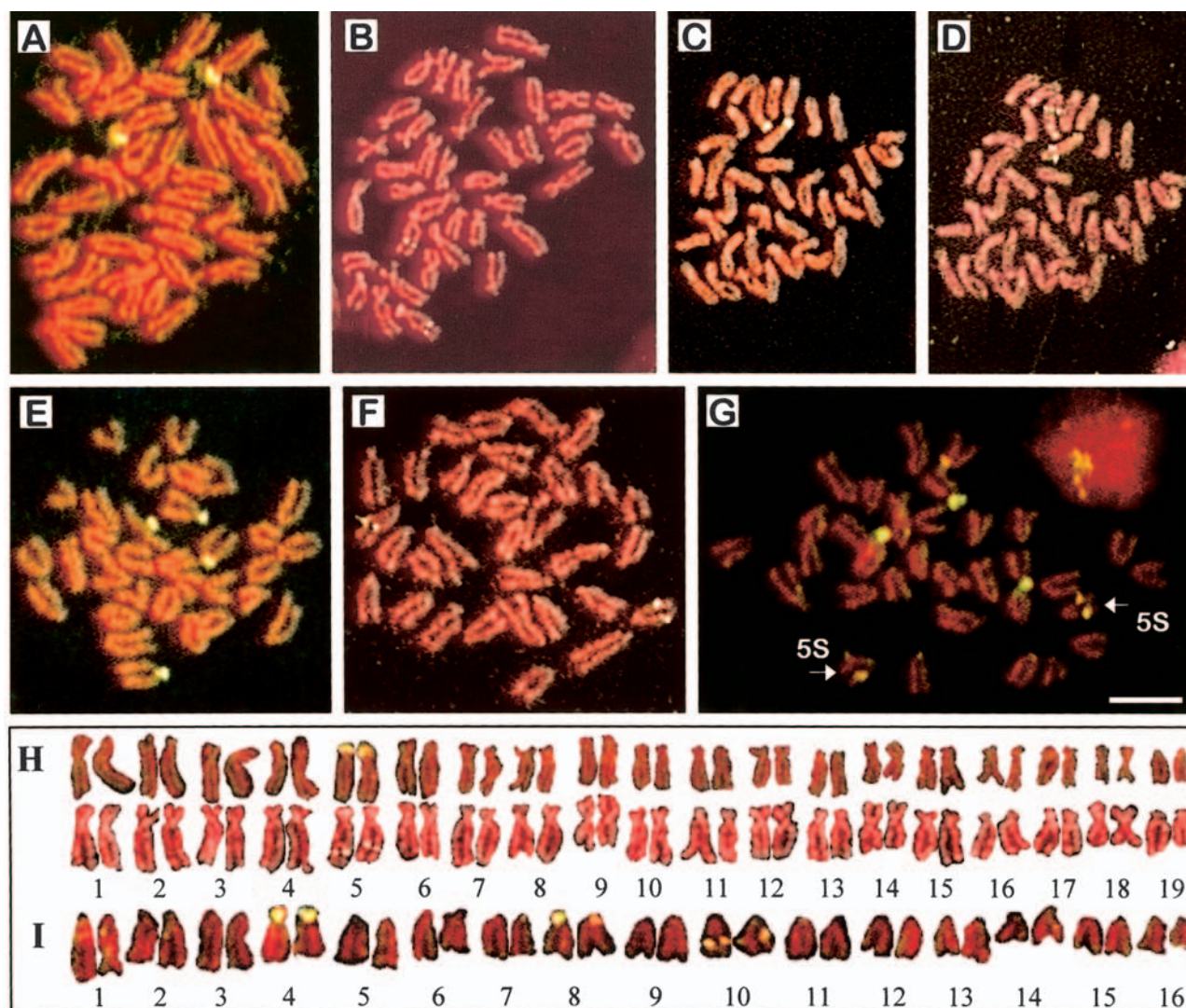


Figure 2. FISH signals and chromosomal location of the major rRNA genes (ITS1) and 5S rRNA genes in *Chlamys farreri* and *Argopecten irradians irradians*. (A) ITS1 in *C. farreri*; (B) 5S rRNA in *C. farreri*; (C and D) ITS1 and 5S on the same metaphase in *C. farreri*; (E) ITS1 in *A. i. irradians*; (F) 5S in *A. i. irradians*; (G) ITS1 and 5S on the same metaphase in *A. i. irradians* with arrows pointing to signals from 5S rRNA; (H) karyotypes of *C. farreri*; and (I) karyotype of *A. i. irradians*. Scale bar = 5 μ m.

To determine whether the 5S and the major rRNA genes are linked on the same chromosome, the two gene families were located on the same metaphase by dual-hybridization. In *C. farreri*, the two genes were located on the same chromosome (Fig. 2C, D), but in different regions. The rRNA gene-bearing chromosome was Chromosome 5 (Fig. 2H), as previously shown. In *A. i. irradians*, the 5S genes were not associated with the two chromosome pairs that carried the major rRNA genes (Fig. 2G). Instead, they were located on Chromosome 10, a telocentric chromosome (Fig. 2H).

The karyotypic characteristics of *C. farreri* and *A. i. irradians* are summarized in Table 3, along with those of 14 other species studied so far. In addition to differences in

haploid number and chromosome morphology, the number and distribution of rRNA gene loci represent another major difference between *C. farreri* and *A. i. irradians*. *C. farreri* had only one chromosome carrying rRNA genes, while *A. i. irradians* had three. *C. farreri* had 38 chromosomal arms, while *A. i. irradians* had 21.

Discussion

Karyotype and rRNA loci

This study provides unambiguous chromosomal mapping of the major (18S–5.8S–28S) and minor (5S) rRNA genes in *Chlamys farreri* and *Argopecten i. irradians*. As far as we can determine, this is the first time that specific genes have

been assigned to chromosomes in the two scallop species. Before this study, chromosomal location of ribosomal RNA genes had been determined using FISH in only one species of scallops—*Aequipecten opercularis* (Insua *et al.*, 1998). In *Nodipecten nodosus* and *Argopecten purpuratus*, two to three Ag-NOR sites were reported at variable locations (Pauls and Affonso, 2000; Gajardo *et al.*, 2002). NORs are supposed to be actively transcribed rRNA sites; however, the number and location of NORs are often variable and may not always provide accurate location of the major rRNA genes (Wang *et al.*, 2004).

This study provides a karyotype for *A. i. irradians*, which has not been described before. The karyotype we describe, 5st + 11t, is identical to that of another species of the same genus, *A. purpuratus*, described by Gajardo *et al.* (2002); it differs, however, from the *A. purpuratus* karyotype of Von Brand *et al.* (1990) (Table 3). As pointed out by Gajardo *et al.* (2002), the karyotype of Von Brand *et al.* (1990) may not be based on proper measurements. The karyotype of *C. farreri* we describe is similar to that described by Komaru and Wada (1985) for the same species.

Modal haploid number and karyotype of Pectinidae

The findings of this study, along with available data from the literature (Table 3), support the hypothesis that the haploid number, $n = 19$, is the modal or ancestral number for Pectinidae. This hypothesis is based on the fact that most

of the species across four genera studied so far have a haploid number of 19 (White, 1954; Beaumont and Zouros, 1991). The other chromosome numbers, $n = 13$ in *Aequipecten opercularis* and $n = 16$ in *Argopecten purpuratus*, were considered as derived primarily because they were less frequent (Beaumont and Gruffydd, 1974; Insua *et al.*, 1998; Gajardo *et al.*, 2002). The assumption that common features are pleisomorphic is not necessarily true. This study provides another and perhaps more convincing evidence that 19 is the modal haploid number of Pectinidae. The finding that *C. farreri* ($n = 19$) has one major rRNA locus and one rRNA-bearing chromosome while *A. i. irradians* ($n = 16$) has two major rRNA loci and three rRNA-bearing chromosomes suggests that the karyotype of *C. farreri* is pleisomorphic and that of *A. i. irradians* is derived. A single locus for rRNA genes is considered as the ancestral state within taxa (Hsu *et al.*, 1975). It is more likely that the one rRNA-bearing chromosome in *C. farreri* splits into three chromosomes than that the three chromosomes consolidate into one. In vertebrates, the major and 5S rRNA genes are rarely located on the same chromosome (Schmid *et al.*, 1987; Lucchini *et al.*, 1993; Mäkinen *et al.*, 1997). The two gene families are found on the same chromosome in the nematode *Meloidogyne arenaria* (Vahidi *et al.*, 1991) and in the gastropod periwinkle *Melarhaphe neritoides* (Colomba *et al.*, 2002).

Given that $n = 19$ is the ancestral haploid number, the

Table 3

Summary of karyotypic data from 16 species of Pectinidae

Species	Haploid number	Arm number	Karyotype ¹							18S	5S	Reference
			m	m/sm	sm	sm/st	st	st/t	t			
<i>Aequipecten opercularis</i>	13	19	2	4	0	0	0	0	7	7q	1q,1q	Insua <i>et al.</i> , 1998
<i>Argopecten i. irradians</i>	16	21	0	0	0	0	5	0	11	4p,8p	10q	This study
<i>Argopecten purpuratus</i>	16	21	0	0	0	0	5	0	11			Gajardo <i>et al.</i> , 2002
	16	28	2	7	0	0	3	0	4			Von Brand <i>et al.</i> , 1990
<i>Chlamys nobilis</i>	16	19	3	0	0	0	0	0	13			Komaru and Wada, 1985
<i>Chlamys glabra</i>	14	—										Rasotto <i>et al.</i> , 1981
<i>Chlamys farreri</i>	19	38	3	0	4	7	4	1	0	5p	5q	This study
	19	38	3	0	1	6	7	2	0			Komaru and Wada, 1985
<i>Chlamys distorta</i>	19	—										Beaumont and Gruffydd, 1974
<i>Chlamys islandica</i>	19	—										Beaumont and Gruffydd, 1974
<i>Chlamys varia</i>	19	—										Beaumont and Gruffydd, 1974
<i>Euvola ziczac</i>	19	37	5	0	6	0	7	0	1			Basoa <i>et al.</i> , 2000
<i>Nodipecten nodosus</i>	19	38	6	0	6	0	7	0	0			Pauls and Affonso, 2000
	19	35	4	0	5	0	7	0	3			Basoa <i>et al.</i> , 2000
<i>Patinopecten yessoensis</i>	19	35	2	1	4	6	3	0	3			Komaru and Wada, 1985
<i>Pecten albicans</i>	19	24	1	1	0	3	0	0	14			Komaru and Wada, 1985
	19	26	0	3	0	0	4	0	12			Ieyama, 1975
<i>Pecten maximus</i>	19	24	2	0	2	0	1	0	14			Beaumont and Gruffydd, 1974
<i>Pecten jacobus</i>	19	—										Rasotto <i>et al.</i> , 1981
<i>Placopecten magellanicus</i>	19	30	0	0	5	0	10	0	4			Xiang <i>et al.</i> , 1993

¹ m = metacentric, sm = submetacentric, st = subtelocentric, t = telocentric; m/sm, sm/st, and st/t are chromosomes overlapping two categories.

karyotype of *C. farreri*, which has the highest number of chromosomal arms (38, Table 3), is probably the closest representative of the ancestral karyotype of Pectinidae. Karyotypes with metacentric or submetacentric chromosomes are considered as more pleisomorphic than those with telocentric and subtelocentric chromosomes (Thiriôt-Quévieux, 1994). We argue that karyotypic changes in Pectinidae started with a karyotype with a haploid number of 19 and 38 arms, similar to that of *C. farreri*, and involved gradual loss of arms by deletion and of whole chromosomes by fusion, leading to all other variant karyotypes in Pectinidae.

Fossil records suggest that the *Aequipecten* group (including *Argopecten*) is a descendent of the *Chlamys* stem group from about 60 million years ago (Waller, 1991). Phylogenetic trees constructed using molecular data are consistent with fossil records and also indicate that the *Aequipecten* group is derived from the *Chlamys* group (Rice *et al.*, 1993; Barucca *et al.*, 2004). Interestingly, three species with reduced chromosome numbers, *Aequipecten opercularis*, *A. i. irradians* and *Chlamys glabra* (Table 3), are found in a single clade distal from the root on the phylogenetic tree of Barucca *et al.* (2004), supporting our conclusion that karyotype of *A. i. irradians* is derived.

Chromosomal rearrangements in Pectinidae

Robertsonian centric fusion has been used to explain chromosome number reductions in Pectinidae. Beaumont and Zouros (1991) postulated that the *Chlamys nobilis* karyotype ($n = 16$), which has 3 metacentric and 13 telocentric chromosomes (Komaru and Wada, 1985), may be derived from the $n = 19$ karyotype, by Robertsonian fusion of three pairs of telocentric chromosomes. Similarly, the karyotype of *Aequipecten opercularis* ($n = 13$), which has 6 metacentric and submetacentric chromosomes and 7 telocentric chromosomes, can also be explained by Robertsonian fusion (Beaumont and Gruffydd, 1974; Insua *et al.*, 1998).

There is no question that centric fusion plays a role in chromosome number reduction in Pectinidae. However, the karyotype of *A. i. irradians* described in this study and that of *A. purpuratus* (Gajardo *et al.*, 2002), both with a reduced haploid number of 16 and without any metacentric chromosomes, cannot be explained by centric fusion alone. They have to involve the loss of chromosome arms after centric fusion. Furthermore, fusion alone cannot explain chromosomal arm losses in scallops with the haploid number of 19, but chromosomal deletion can (Table 3).

We argue that chromosomal deletion is a major feature of karyotypic changes in Pectinidae, as indicated by the wide range of chromosomal arms (19–38, Table 3) and DNA contents (2.23–3.28 pg per diploid genome) (Rodríguez-Juíz *et al.*, 1996; Gonzáles-Tizón *et al.*, 2000; Thiriôt-

Quévieux, 2002). The four species with reduced haploid numbers (13 or 16) have an average of 20 chromosome arms, ranging from 19 to 21 (Table 3). On the other hand, the four species with the ancestral haploid number ($n = 19$) have an average of 30 chromosome arms, ranging from 24 to 38. The loss of chromosome arms is likely caused by chromosomal deletion, rather than relocation, as the reduction in arm number in *Aequipecten opercularis* (by 20.8%) corresponds to a similar reduction in DNA content (by 21.2%), using *Pecten maximus* as a reference species (Rodríguez-Juíz *et al.*, 1996). More data on DNA content from other species are needed to verify the correlation.

Results of this study also suggest that chromosomal translocation and duplication may play a role in karyotypic evolution in Pectinidae. The finding that the major and minor rRNA genes are located on the same chromosome in *C. farreri* but on two different chromosomes in *A. i. irradians* can be explained by translocation. The fact that *A. i. irradians* has two loci for the major rRNA genes while *C. farreri* has only one points to chromosomal duplication, possibly through unequal translocation.

We speculate that the karyotype of *A. i. irradians* evolved from an ancestral karyotype of $n = 19$, similar to that of *C. farreri*, possibly involving the following steps: (1) loss of the chromosomal arms and emergence of telocentric chromosomes; (2) fusion of telocentric chromosomes; (3) deletion of chromosomal arms after fusion; and (4) translocation and duplication of the major rRNA loci. Further studies involving more species may improve our understanding of karyotypic changes in Pectinidae.

Evolutionary implications: Is the ancestral karyotype tetraploid?

Assuming that the karyotype of *C. farreri* represents the ancestral/modal form ($n = 19$, 38 arms), evolution leading to the *Aequipecten* ($n = 13$) and *Argopecten* ($n = 16$) is accompanied by significant loss of chromosomal arms (by about 50%). If this is true, it raises the question of why scallops can tolerate so much chromosomal loss. The most obvious explanation is that the ancestral karyotype, $n = 19$, may be polyploid. Polyploid species are known to tolerate considerable loss of chromosomes (White, 1978). We hypothesize that the ancestral karyotype of Pectinidae ($n = 19$) is tetraploid and evolved by genome duplication from an ancestral bivalve with a haploid number of about 10, similar to that of Ostreidae ($n = 10$). During or soon after the genome duplication, one pair of chromosomes was lost, leading to a relatively stable karyotype of $n = 19$. Since most bivalves studied so far have a haploid number of 19 chromosomes (Nakamura, 1985; Rodríguez-Juíz *et al.*, 1996; Thiriôt-Quévieux, 2002), it is possible that all bivalve groups with a modal haploid number of 19 are tetraploid and derived from a common diploid or triploid

ancestor. Bivalve species with variant haploid numbers between 13 and 16 represent a triploid state, either derived from the diploid species or formed through chromosome reduction from the tetraploid form by a process similar to that found in Pectinidae.

Ostreidae, with a haploid number of 10 in most member species studied so far (Nakamura, 1985; Thiriou-Quévieux, 2002), may represent the closest relative of the diploid ancestor. Triploid and tetraploid oysters are fully viable and phenotypically indistinguishable from diploids (Stanley *et al.*, 1981; Guo *et al.*, 1996). Triploid and tetraploid oysters can easily tolerate the loss of two chromosomes in one generation without obvious deleterious effects, while diploids can rarely tolerate the loss of one (Wang *et al.*, 1999). Polyploid oysters can lose chromosomes *de novo* and can even revert from triploidy to diploidy or from tetraploidy to triploidy (Allen *et al.*, 1997). Tetraploids in the Pacific oysters produce significant proportions of aneuploid gametes with chromosome losses due to multivalent formation (Guo and Allen, 1997b). Therefore, genome duplication from $n = 10$ to $n = 19$ is theoretically possible. A quick examination of limited data on DNA content seems to support the genome duplication hypothesis. DNA content per diploid nucleus ranged from 1.82 to 2.33 pg in Ostreidae, compared to 2.83–3.94 pg in bivalves with a haploid number of 19 chromosomes (Rodríguez-Juíz *et al.*, 1996; González-Tizón *et al.*, 2000; Thiriou-Quévieux, 2002). Minor deviations in DNA content and chromosome numbers can be explained by chromosomal losses or gains, and the pattern is in general agreement with the genome duplication hypothesis.

In summary, we suggest that at least one genome duplication event has occurred during the evolution of bivalves. The tetraploid hypothesis is primarily based on the findings that (1) the ancestral karyotype of Pectinidae ($n = 19$) can tolerate significant amount of chromosome loss, leading to the formation of *A. i. irradians* (this study); (2) most bivalves have a haploid number of 19 (Nakamura, 1985; Rodríguez-Juíz *et al.*, 1996; Thiriou-Quévieux, 2002), while Ostreidae has a modal haploid number of 10; and (3) there is a general correlation between chromosome number and DNA content, and variations can be explained by chromosomal deletion, fusion, translocation, and limited duplication, as shown for Pectinidae in this study. Although our hypothesis on genome duplication is somewhat speculative, it is the most logical explanation of available data and provides a theoretical framework for further testing and analysis. Phylogenetic relationships among most bivalve groups are not well understood (Schneider, 2001). Recently, molecular studies have produced phylogenetic trees that are in general agreement about the relationship among most major groups (Adamkewicz *et al.*, 1997; Giribet and Wheeler, 2002; Giribet and Distel, 2003). Supporting our genome duplication hypothesis, the basal positions of the

phylogenetic trees are dominated by species with low haploid numbers, including Ostreidae ($n = 10$) and Solemya ($n = 11$); the derived branches are dominated by species with $n = 19$; and species with $n = 14$ lie in between. Two of the three trees are rooted with Polyplacophora (Adamkewicz *et al.*, 1997; Giribet and Distel, 2003), which has a modal haploid number of $n = 12$ (Patterson, 1969) and is a close relative of the bivalves. It is possible that Polyplacophora and Bivalvia share a common ancestor which, after genome duplication, gave rise to scallops and clams with haploid numbers of 19.

Clearly, significant chromosomal rearrangements occurred during the evolution of Pectinidae and Bivalvia. Whether these changes are the driving forces for speciation or merely byproducts of other evolutionary events remains unclear. It is possible that scallops with reduced chromosome numbers evolved as a result of chromosomal rearrangements, but supporting data are yet to be collected. *C. farreri* and *A. i. irradians* can cross-fertilize with a success level of up to 90%, and the larvae can survive for 12 days (Chen *et al.*, 1991), but no viable hybrids have been reported. It is not known if the postzygotic barrier to hybridization is chromosomal or genic. This study does demonstrate the usefulness of FISH analysis and the need for similar studies in more species of pectinids and other bivalves.

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