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ORIGINAL PAPER

Chromosome rearrangements in Pectinidae (Bivalvia: Pteriomorphia) implied based on chromosomal localization of histone H3 gene in four scallops

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Abstract Chromosomal structural rearrangement in four scallops, *Chlamys farreri* ($n = 19$), *Patinopecten yessoensis* ($n = 19$), *Chlamys nobilis* ($n = 16$) and *Argopecten irradians* ($n = 16$), was studied by fluorescence in situ hybridization using histone H3 gene probes. The results show that histone H3 gene sites differ strikingly with regard to number, location, and intensity among, or even within these species. For example, two histone H3 gene loci were detected on the metaphase chromosomes of *P. yessoensis*, while one locus was found in the others. In *P. yessoensis*, differing intensities of hybridization signals were detected between homologues 5 and 11, and within homologue 11. These data suggest that the histone H3 gene is a qualified chromosome marker for the preliminary understanding of the historical chromosomal reconstructing of the Pectinidae family. The variable distribution patterns of the histone H3 gene suggest that gene duplication/diminution as well as chromosome rearrangements by inversion and translocation may have played important roles in the genomic evolution of Pectinidae. We also compiled our present results with former published data regarding the chromosome mapping of rDNAs in species of the Pectinidae family. Such comparative chromosomal mapping should improve our understanding of historical chromosomal reconstructions of modern-day scallops.

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Introduction

The family Pectinidae consists of more than 300 living species. Since the late 18th century, a wide range of systematic studies have been conducted in terms of morphological features (Korobkov 1960; Waller 1991, 1993) and molecular phylogeny (Matsumoto and Hayami 2000; Barucca et al. 2004). However, the comparative cytogenetic investigations among Pectinidae species have been poorly presented, and are rarely informative in terms of phylogenetic estimation. As documented by numerous cytogenetic data over the past years (e.g. Komaru and Wada 1985; Pauls and Affonso 2000), among the 16 scallops examined, five showed haploid chromosome numbers ($n = 13$–16) and deviated from 19 (Insua et al. 1998; Wang and Guo 2004), which is now considered to be the most frequent number among bivalves (Thiriot-Quiévreux 2002). Moreover, deviation in chromosome morphologies has been revealed in terms of the fundamental number (NF), which has been shown to range, for example, from 24 to 38 (Wang and Guo 2004), so their karyotypes are actually highly variable and not truly conservative. The exact evolutionary mechanism that is responsible for the karyotypic variation in Pectinidae is unknown, but at least two ancestral karyotypes have been proposed, $2n = 20$ (Gajardo et al. 2002) and $2n = 38$ (Wang and Guo 2004), and the major trends of these karyotypes are

strongly controversial. This diversity suggests that chromosomal rearrangements played significant roles in the development of modern chromosomal variation, which in turn highlights the need for deeper insight into the chromosome-reconstructing events in this group.

The most frequently used technique for cytogenetically investigating interspecific comparisons is fluorescence in situ hybridization (FISH). FISH allows researchers to gain a better resolution for the identification of chromosomes and makes it possible to track chromosomal rearrangements at a minute scale (Hirai et al. 1996; Weiss and Maluszynska 2000; Ran et al. 2001). The easiest and most successful application of in situ hybridization concerns the localization of moderately long sequences that are repeated more than 100 times at one place in the genome (Macgregor and Varley 1983). In bivalves, most comparative studies on karyotypes have focused on repetitive sequences such as ribosomal RNA genes (rDNAs). Consequently, comparative studies on diverse bivalves have shown that chromosome structures are incredibly dynamic in terms of number and location of rDNAs (Insua and Méndez 1998; Insua et al. 2001; Wang et al. 2004). Wang and Guo (2004) conducted a FISH-detected major and minor rDNA patterning study in *Chlamys farreri* and *Argopecten irradians* and postulated that chromosomal translocation and duplication may play a dominant role in the karyotypic evolution of Pectinidae.

Histone H3 is among the most conserved eukaryotic proteins (Miller et al. 1993). Most histone H3 genes are repeatedly organized into clusters (Albig et al. 1996), which makes them an ideal chromosomal marker for tracking historical and ongoing karyotypic repatterning. Consequently, histone gene mapping with the FISH technique has been conducted in many vertebrates, particularly humans, mice and fish (Tripputi et al. 1986; Pendás et al. 1994; Drabent et al. 1995; Wang et al. 1996). In bivalves, however, little knowledge is available concerning sequence information or the cytogenetic characteristics of histone genes. In the present study, we developed the histone H3 gene as a new FISH-detected marker, and examined the redistribution of chromosomal elements among four Chinese commercial scallops: *C. farreri* ($n = 19$, NF = 38), *C. nobilis* ($n = 16$, NF = 19), *Patinopecten yessoensis* ($n = 19$, NF = 35), and *A. irradians* ($n = 16$, NF = 21). We also compiled our data with published chromosomal rDNA mapping data to evaluate the feasibility of studying chromosomal rearrangements within the Pectinidae family.

Materials and methods

Scallop materials

The zhikong scallop (*C. farreri*), noble scallop (*C. nobilis*) and bay scallop (*A. irradians*) used in this study were from an aquacultural hatchery in Penglai, Shandong Province, China. The Japanese scallop (*P. yessoensis*) was from an aquacultural hatchery in Weihai, Shandong Province, China.

DNA preparation, PCR, cloning and sequencing

DNA extraction was carried out by the standard phenol-chloroform procedure using adductor muscle (Sambrook et al. 1989). The purity and quality of DNA were checked by 0.8% agarose gel electrophoresis. PCR amplification of the histone H3 gene was performed using a pair of primers (forward: 5′-CGTAAATC-CACTGGAGGCAAGG-3′; reverse: 5′-GGATGGCG CACAGGTTGGTGTC-3′), designed from the H3 nucleotide sequences of *Pecten jacobaeus* and *Mimachlamys varia* retrieved from GenBank (AY070153-AY070154). The amplification mixture contained 100 ng genomic DNA, 0.2 μM of each primer, 200 μM of dNTP and 1U Taq polymerase (Promega) in a total volume of 20 μl. Amplification was performed with a PTC-100 thermocycler (MJ Research, USA). Cycling conditions were as follow: 5 min at 94°C (denaturation); 30 cycles of 1 min at 94°C, 40 s at 66°C, and 1 min at 72°C; and a final 5 min at 72°C (extension). PCR products of the expected size (316 bp) were cloned into the plasmid pMD18-T (Takara Inc., Dalian, China), and sequenced on an ABI Prism 377 DNA sequencer. The sequence results were compared with the GenBank database. The nucleotide sequences have been deposited in the NCBI database under accession numbers DQ407913-DQ407916.

Chromosome preparation and FISH

Following treatment with colchicine (0.01%)for 2 h at room temperature, the trophore larvae were exposed to 0.075 M KCl solution for 30 min and then fixed three times (15 min each) in fresh ethylalcohol/glacial acetic acid solution (3:1). After being treated with 50% acetic acid, the fixed larvae were dissociated into a cell suspension, and then dropped onto hot-wet slides and air-dried.

In FISH experiments, we used recombinant clones as probes, which were labeled by PCR with biotin-16-dUTP. Chromosome spreads were pretreated with 100μg/ml DNase-free RNase A in 2× SSC for 1 h at

37°C, and then treated with 0.005% pepsin in 10 mM HCl for 10 min at 37°C. Chromosome preparations were denatured in a mixture containing 70% formamide and 2× SSC at 72°C for 2 min, dehydrated with a chilled ethanol series (70, 90, and 100%; 5 min each), and then air-dried. Slides were then incubated with 20 μl of denatured hybridization mix (5 ng/μl probe, 10% dextran sulphate, 250 ng/μl salmon sperm DNA, 50% deionized formamide in 2× SSC, 80°C for 5 min and cooled immediately) for 16 h at 37°C in a moist chamber. After hybridization, slides were washed three times (5 min each) in 50–60% formamide in 2× SSC at 45°C, three times (5 min each) in 1× SSC at 45°C, once for 5 min in 2× SSC at RT. Hybridized probes were detected with fluorescein-labeled avidin DCS (Vector Laboratories). Chromosomes were counterstained with 1.5 μg/ml propidium iodide (PI) in antifade solution (Vector Laboratories). Slides were observed using a Nikon Eclipse-600 epifluorescence microscope equipped with a CCD camera. The signals were collected using appropriate filter sets and LUCIA software (Laboratory Imaging).

Results

We examined at least 50 good chromosome spreads for each species. Even without additional optimizing procedures for the hybridization and washing conditions, FISH yielded a high signal/background ratio on the spreads. Our results show that the histone H3 gene was clustered around a single locus on the chromosomes of *C. farreri, C. nobilis* and *A. irradians*, and was mapped at two different loci in *P. yessoensis*. The localization of the histone H3 gene on the chromosomes exhibited significant polymorphism among the four scallops studied: in *A. irradians*, it was mapped to the largest telocentric chromosome that could be assigned to pair 1 (Wang and Guo 2004), located about one-third of the way from the centromere to the telomere (Fig. 1a, e); in *C. nobilis*, at the telomeric region of a telocentric chromosome pair 7 (Fig. 1b, f) (Komaru and Wada 1985); in *C. farreri*, on the telomeric region of the short arm of a large submetacentric chromosome that could be assigned to pair 2 (Fig. 1c, g) (Wang and Guo 2004); and in *P. yessoensis*, on two different pairs of chromosomes, one at an interstitial site on the short arm, about half the arm-length away from the centromere of a large submetacentric chromosome (pair 5), and the other about halfway from the centromere to the telomere of a small subtelocentric pair (pair 11) (Fig. 1d, h).

The different intensities of the hybridization signals between chromosome pairs 5 and 11, and within the two homologues of pair 11, were clearly obvious in *P. yessoensis*. By examining 50 metaphases, we found that about 80% displayed brighter signals in chromosome pair 11 than in 5. Up to 57% of the metaphase spreads showed a difference in the intensities of histone H3 gene signals between the two homologues of pair 11.

Discussion

FISH mapping of histone H3 gene in four scallops

Based on the chromosomal localization of the histone H3 gene among the four species, the chromosomes carrying histone H3 gene varied from submetacentrics to telocentrics, and the location of the histone H3 gene differed from the interstitial to the telomeric region. Particularly, in *P. yessoensis*, two loci were found on different homologous pairs, while only one locus was found in the other three species examined. The variable distribution patterns of the histone H3 gene suggest that gene duplication/diminution, as well as chromosome rearrangements by inversion and translocation, may have played important roles in the genomic evolution of Pectinidae.

The phenomenon of different intensities for hybridization signals between chromosome pairs 5 and 11 in *P. yessoensis* is very similar to that of FISH-detected major rRNA genes in the razor clam *Solen marginatus* (Fernández-Tajes et al. 2003) as well as the histone genes in *Mytilus galloprovincialis* (Eirín-López et al. 2002, 2004). A possible explanation for the mechanism responsible for this phenomenon might be gene duplication followed by non-homologous recombination or a translocation event. Subsequently, small rearrangements would result in the gradual breakup, dispersal or shrinking of one pair, and, as a result, different chromosomal pairs would contain different copy numbers of the histone H3 gene. This explanation corresponds well with the distinct intensities of FISH signals we observed. Within the two homologues of pair 11, 57% of the metaphase spreads showed different intensities for the hybridization signals, which is consistent with the results reported by Insua et al. (1998). A likely cytogenetic explanation could be unequal crossover during meiosis, or unequal exchanges between mitotic sister chromatin within the histone H3 gene repetitive sequences of homologue 11. This mechanism might result in one of these two sequences becoming too small to be visualized by

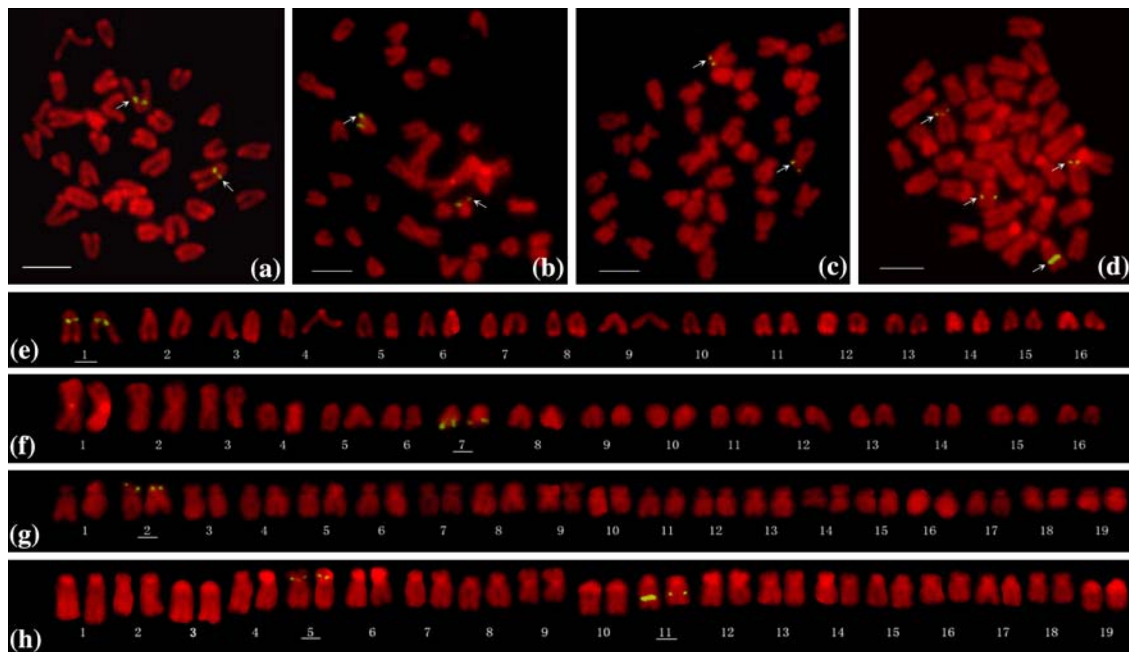

Fig. 1 FISH signals and chromosomal location of histone H3 gene in (**a**), *A. irradians*; (**b**), *C. nobilis*; (**c**), *C. farreri*; (**d**), *P. yessoensis*. Arrows indicate histone H3 gene sites. (**e**), karyotype of *A. irradians*; (**f**), karyotype of *C. nobilis*; (**g**), karyotype of *C. farreri*; (**h**), karyotype of *P. yessoensis*. Chromosome numbering is based on descending order of relative length and the chromosomes carrying the histone H3 gene are underlined

FISH, while the amplified one rapidly expands in size and becomes much more cytogenetically detectable than its counterpart.

There is possibility that homomorphic variants as H3.3 could be revealed by FISH using a probe designed from a canonical H3 gene due to the subtle differences between them at the protein level. However, little is known about histone variants in bivalves. According to our analysis of nucleotide sequences of H3.3 (AY916802) and H3 (DQ067446) in the Manila Clam *Venerupis philippinarum* retrieved from GenBank, identities between these two genes are less than 80%. In our study, post-hybridization washes were carried out at a stringency of ~80% to 86%, thus these signals should mostly result from canonical H3 genes.

Karyotype evolution implied based on the localization of histone H3 and rRNA genes in Pectinidae

In scallops, FISH analysis with tandem moderately repetitive rRNA genes proved to be very effective for preliminarily understanding genomic reconstruction at the cytogenetic level (Wang and Guo 2004). Chromosome mapping of the histone H3 gene has turned out to be another qualified chromosomal marker. The variable distribution patterns make it very informative and even more efficient than rDNA when proposing evolutionary chromosome changes in Pectinidae. We compiled our present data with previously published chromosome mapping data for major and minor rRNA genes in Pectinidae (Table 1).

(1) Histone H3 and major and minor rRNA genes were localized on one or two chromosome pairs in each genome, and the number of these markers was found to be unrelated to the haploid number of the corresponding scallops. Regarding histone H3 genes, *P. yessoensis* ($n = 19$) bears duplicate loci, while the other three, with haploid numbers ranging from 16 (*C. nobilis* and *A. irradians*) to 19 (*C. farreri*), bear only one locus. Major rRNA genes are clustered in one locus in *C. farreri* and *A. opercularis* ($n = 13$), while for the other three scallops with haploid numbers ranging from 16 (*A. irradians*) to 19 (*Hinnites distortus* and *P. yessoensis*), they were in two loci. With regard to minor rRNA genes, they are clustered in two loci on the same chromosome in *A. opercularis*, while only one locus has been found in the other species that possess high haploid numbers.

(2) The localization of the three markers is highly variable among species. Major rRNA genes have a tendency to be localized at the distal regions of chromosomes, but they vary in position from long arms (*A. operculari*) to short arms (*P. yessoensis,*

Table 1 Summary of the location of the histone H3 and rRNA genes in Pectinidae

Species	n	histone H3 multigene family				Major rDNA multigene family				Minor rDNA multigene family			
		No.	Location [a]	Type [b]	References	No.	Location [a]	Type [b]	References	No.	Location [a]	Type [b]	References
Hinnites distortus	19	unknown				2	(peri)centromeric (peri)centromeric	st st	López-Piñón et al. (2005)	1	pericentromeric, q	st	López-Piñón et al. (2005)
Patinopecten yessoensis	19	2	interstitial, p interstitial, q	sm st	In this study	2	telomeric, p telomeric, p	sm sm	unpublished	unknown			
Chlamys farreri	19	1	telomeric, p	sm	In this study	1	telomeric, p	sm/st [c]	Wang and Guo (2004)	1	interstitial, q	sm/st [c]	Wang and Guo (2004)
Chlamys nobilis	16	1	telomeric, q	t	In this study	unknown				unknown			
Argopecten irradians	16	1	interstitial, q	t	In this study	2	telomeric, p telomeric, p	st st	Wang and Guo (2004)	1	interstitial, q	t	Wang and Guo (2004)
Aequipecten opercularis	13	unknown				1	telomeric, q	t	Insua et al. (1998)	2	interstitial [e] interstitial [e]	m/sm [d] m/sm [d]	Insua et al. (1998)

[a] p: short arm; q: long arm

[b] m = metacentric, sm = submetacentric, st = subtelocentric, t = telocentric; m/sm and sm/st are chromosomes that overlap two categories

[c, d] represents the same chromosome, respectively

[e] represents the same arm

C. farreri and *A. irradians*). Minor rRNA genes are mostly localized in interstitial regions of chromosomes. However, chromosomes that carry this gene are morphologically distinct, and vary from metacentric (*A. opercularis*), to acrocentric (*H. distortus* and *C. farreri*), to telocentric (*A. irradians*).

(3) Our data regarding the histone H3 gene locus and previous research on chromosome mapping of major and minor rRNA genes (Wang and Guo 2004), along with the phenomenon that a wide range of species in Pectinidae share a conservative diploid number of 38 (Thiriot-Quiévreux 2002), we assume that chromosomal translocation (Wang and Guo 2004) and inversions, rather than Robertsonian translocations (which explain rapid changes in chromosome numbers), may be the major mechanisms by which Pectinidae species experienced chromosomal evolution. At present, chromosome mapping of these markers is not sufficient to infer karyotypic relationships among modern-day scallops. Wang and Guo (2004) found that major and minor rRNA genes are located on the same chromosome in *C. farreri* but on three different chromosomes in *A. irradians*, and consequently suggested that the karyotype of *C. farreri* is pleisomorphic, while that of *A. irradians* is derived. It may be questionable as the localization of major and minor rRNA genes on different chromosomes is common in other bivalves, such as *A. opercularis, H. distortus, M. galloprovincialis* and *M. edulis* (Insua et al. 1998, 2001; López-Piñón et al. 2005). Variations with these markers are more suited to discussing the sort of chromosomal changes involved in genome evolution, rather than in accurately reflecting the evolutionary dynamics of each constituent in a karyotype. Most importantly, though these markers reflect numerous chromosome reconstructions, it is impossible to use them to formulate a major historical trend of chromosomal evolution or to therefore infer the ancestral karyotype of Pectinidae.

Summary

In this study, we developed the histone H3 gene as a marker for the chromosomal research of scallops. Combined with the FISH technique, it provided a good opportunity to examine current gene dispersal, and thus to deduce historical chromosome repatterning in the genomic evolution of scallops. Our data, combined with those from previous reports on chromosome

mapping of major and minor rRNA genes, suggest that gene duplication/diminution, as well as chromosome rearrangements by inversions and translocation, may have played important roles during chromosome evolution in Pectinidae. Recognizing the events of chromosome reconstruction also sheds light on the need to expand our research on chromosomal macromutations to encompass a wider range of bivalve species.

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