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E-mail:xbywb@ouc.edu.cn

Microsatellite-Centromere Mapping in Japanese Scallop (*Patinopecten yessoensis*) Through Half-Tetrad Analysis in Gynogenetic Diploid Families

LI Qi[1), *], QI Mingjun[1)], NIE Hongtao[2)], KONG Lingfeng[1)], and YU Hong[1)]

1) *Key Laboratory of Mariculture of Ministry of Education*, *Ocean University of China*, *Qingdao* 266003, *P. R. China*
2) *College of Fisheries and Life Science*, *Dalian Ocean University*, *Dalian* 116023, *P. R. China*

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Abstract Gene-centromere mapping is an essential prerequisite for understanding the composition and structure of genomes. Half-tetrad analysis is a powerful tool for mapping genes and understanding chromosomal behavior during meiosis. The Japanese scallop (*Patinopecten yessoensis*), a cold-tolerant species inhabiting the northwestern Pacific coast, is a commercially important marine bivalve in Asian countries. In this study, inheritance of 32 informative microsatellite loci was examined in 70-h D-shaped larvae of three induced meiogynogenetic diploid families of *P. yessoensis* for centromere mapping using half-tetrad analysis. The ratio of gynogenetic diploids was proven to be 100%, 100% and 96% in the three families, respectively. Inheritance analysis in the control crosses showed that 51 of the 53 genotypic ratios observed were in accordance with Mendelian expectations at the 5% level after Bonferroni correction. Seven of the 32 microsatellite loci showed the existence of null alleles in control crosses. The second division segregation frequency (y) of the microsatellite loci ranged from 0.07 to 0.85 with a mean of 0.38, suggesting the existence of positive interference after a single chiasma formation in some chromosomes in the scallop. Microsatellite-centromere distances ranged from 4 cM to 42 cM under the assumption of complete interference. Information on the positions of centromeres in relation to the microsatellite loci will represent a contribution towards the assembly of genetic maps in the commercially important scallop species.

Key words *Patinopecten yessoensis*; gynogenetic diploid; microsatellite; half-tetrad analysis; microsatellite-centromere mapping

1 Introduction

The Japanese scallop *Patinopecten yessoensis* is a cold-tolerant species inhabiting coastal waters of the northern islands of Japan, the northern part of the Korean Peninsula, and Russian Primorye, Sakhalin and Kurile islands. The scallop was introduced into China from Japan in 1982 to increase the scallop cultivation in the northern part of the Chinese coast. As it is larger in size thus commands a higher market price than the native zhikong scallop (*Chlamys farreri*), *P. yessoensis* was quickly accepted by scallop farmers in northern China, and its aquaculture expanded rapidly. In 2012, world production of this species was 318081 tons (FAO, 2014). With the expansion of culturing operations of the scallop, some problems have been encountered, including mass mortality, poor growth and poor seed production (Li and Xue, 2005). To improve the Japanese scallop industry, some genetic studies have been carried out (Zhao *et al*., 2006; He *et al*., 2012; Ding *et al*., 2015).

Over the past decade, significant progress has been made in constructing genetic maps for aquatic animals with the increasing interest in genomics. Gene mapping is an essential requisite for the identification and mapping of quantitative trait loci (QTL), marker-assisted selection (MAS), evolutionary studies by syntenic characterization, positional cloning of genes, and contig assembly (Smith *et al*., 2002). Gene-centromere mapping is an essential prerequisite for our understanding of the composition and structure of genomes. Genetic maps have been published for the Japanese scallop using amplified fragment length polymorphism (AFLP) and microsatellite markers (Chen *et al*., 2012; Xu *et al*., 2008); however, there is still no marker-centromere (M-C) mapping documented in the scallop species.

Marker-centromere mapping estimates have been reported in many fish species such as salmonids (Lindner *et al*., 2000), carps (Zhu *et al*., 2013; Feng *et al*., 2015) and catfish (Poompuang and Sukkorntong, 2011). They have also been reported in several shellfish species including the Pacific oyster (Hubert *et al*., 2009), Pacific abalone (Nie *et al*., 2012), blue mussel (Besumont and Fairbrother, 1995) and Zhikong scallop (Nie *et al*., 2013). In many more organisms the centromeres can be localized with half-tetrad analysis. Half-tetrad analysis is a power-

* Corresponding author. Tel: 0086-532-82031622
E-mail: qili66@ouc.edu.cn

ful tool for mapping genes and understanding chromosomal behavior during meiosis (Thorgaard *et al.*, 1983). In fishery animals, gynogenetic diploids or triploids produced by the inhibition of the second polar body release provide a mean to analyze meiosis II half-tetrads. The recombination rate between the gene or marker and the centromere can be estimated from the frequency of recombinant heterozygous genotype in the half-tetrad progeny of the heterozygous mother. The proportion of heterozygous progeny is a measure of the frequency of second division segregation. Thus, the marker-centromere distance can be estimated by using an appropriate map function.

In the present study, we produced three families of gynogenetic Japanese scallop by inhibiting the second polar body in gynogenetically activated eggs, and used microsatellite genotyping data to estimate M-C map distances. This work will provide useful information for gene mapping in this economically important marine bivalve species.

2 Materials and Methods

2.1 Experimental Families and Genomic DNA Extraction

Mature cultured Japanese scallops were collected in early March 2012 from the coast of Penglai, Shandong Province, China. Eggs and sperms were obtained by artificially inducing spawning with the stimulation of dryness and raising water temperature. Suspensions of sperm and egg were prepared at concentrations of 1.0×10^7 sperm mL^{-1} and 2.0×10^4 egg mL^{-1}, respectively. Three families (A, B and C) were created using eggs and sperm from a single female and male each case. To produce gynogenetic diploids, eggs were inseminated with ultraviolet (UV)-irradiated sperm followed by inhibition of second polar body with cytochalasin B (0.5 $\mu g\,mL^{-1}$; CB) treatment at 45 min postinsemination for 20 min. The eggs inseminated with normal sperms were used as diploid control. At 70 h after insemination, samples of D-shaped larvae from the treatment and control groups were collected, and transferred into 1.5-mL sterile microfuge tubes. In the previous study, the D-shaped larvae, which did not occur in the haploid group, but were observed in the CB-treated gynogenetic diploid groups, were confirmed to be gynogenetic diploids (Li *et al.*, 2000). After gentle centrifugation at 3000 $r\,min^{-1}$ for 5 min, seawater was removed, and then the larvae were preserved in 100% ethanol at 4℃.

Larval DNA was prepared by the Chelex extraction method as previously described (Li and Kijima, 2006), and parental DNA was extracted from muscle tissues using a standard phenol/chloroform extraction procedure.

2.2 Microsatellite Genotyping

A total of 75 microsatellite markers developed in *P. yessoensis* (An *et al.*, 2005; Chen *et al.*, 2009; Sato *et al.*, 2005; Sun *et al.*, 2007; Wang *et al.*, 2009; Zhao *et al.*, 2006) were evaluated for M-C mapping suitability through PCR amplification and genotyping of parents and eight control progeny. Markers were selected from this set for estimation of M-C recombination rate based on amplification efficiency, scoring clarity and informativeness. PCR was performed in a 10 μL^{-1} volume containing 0.25 U *Taq* DNA polymerase (Takara), 1× PCR buffer, 0.2 mmol L^{-1} dNTP mix, 0.5 $\mu mol\,L^{-1}$ of each primer set, 1.6 mmol L^{-1} $MgCl_2$, and about 100 ng template DNA. PCR were performed using a PCR thermal cycler as follows: 3 min at 94℃; seven cycles of 1 min at 94℃, 30 s at the optimal annealing temperature, and 30 s at 72℃; 38 cycles of 30 s at 94℃, 30 s at annealing temperature, 30 s at 72℃, and a final extension of 5 min at 72℃. Amplification products were resolved *via* 6% denaturing polyacrylamide gel, and visualized by silver staining. The 70-h D-shaped larvae from the treatment and control groups of three families were genotyped individually.

2.3 Estimation of M-C Distances

To confirm Mendelian inheritance observed genotype proportions in control progeny were tested against the expected Mendelian segregation ratios (1:1, 1:2:1, and 1:1:1:1) using χ^2 analysis. The sequential Bonferroni correction (Rice, 1989) was considered for multiple tests. M-C recombination rate (second division segregation frequency, y) was estimated from the frequency of heterozygous recombinant genotype in the meiotic gynogenetic progeny for the segregating loci. Homozygosity induced by one generation of gynogenesis is defined as the fixation index, F, and was estimated by $(1-y)$ (Allendorf and Leary, 1984). The y values were converted to map distances (x) in centimorgans between the microsatellite locus and centromere in three ways. In the first calculation, where $x=y/2$, complete interference is assumed (*i.e.*, one recombinational exchange completely inhibits additional crossovers). The second method for calculating map distance is based on the Kosambi equation

$$x=[\ln(1+y)-\ln(1-y)]\times100/4\,,$$

which assumes 50% interference (Kosambi, 1943). The third method is in accordance with Haldane equation

$$x=-[\ln(1-y)]\times100/2\,,$$

which assumes zero interference (Haldane, 1919).

3 Results

3.1 Mendelian Segregation

Thirty-two microsatellite loci heterozygous in the female parents were selected from 75 microsatellites and individually amplified in each family. The parental genotypes and observed genotype numbers of control and gynogenetic offspring at the loci are shown in Table 1. Of the 53 genotypic ratios observed, 51 were in accordance with Mendelian expectations at the 5% level after sequential Bonferroni correction.

3.2 Verification of Meiotic Gynogenesis

The male parent carried 14 unique alleles at 9 loci in family A, 12 unique alleles at 10 loci in family B, and 13 unique alleles at 10 loci in family C (Table 1). The unique alleles were absent in the counterpart female, and thus were useful to examine whether male parent contributed to the offspring genome.

The ratio of gynogenesis was proven to be 100.0%, 100.0% and 96.0% in the three families, respectively. None of the 70-h D-shaped larvae sampled was homozygous at all loci examined. This indicated that these larvae were not haploids. They were true gynogenetic diploids and could be used for M-C mapping.

Table 1 Genotypic proportions in gynogenetic and control progeny at informative microsatellite loci

Locus	Family	Parent genotype	Genotype of control progeny				Genotype of Gynogenetic progeny			χ^2	P	y
KMY90	B	♀ A/B	A/A	A/B	A/C	B/C	A/A	A/B	B/B	5.897	0.117	0.409
		♂ A/C	6	12	3	8	12	18	14			
KMY102	C	♀ A/B	A/A	A/B	A/C	B/C	A/A	A/B	B/B	7.143	0.067	0.565
		♂ A/C	4	11	3	10	5	26	15			
KMY139	B	♀ A/B	A/null	B/(B or null)	A/B		A/A	A/B	B/B	11.121	0.004	0.475
		♂ B/null	3	11	16		4	19	17			
	C	♀ A/B	A/B	A/C	B/B	B/C	A/A	A/B	B/B	0.857	0.836	0.214
		♂ B/C	7	6	6	9	9	9	24			
KMY158	A	♀ A/B	A/A	A/B	B/B		A/A	A/B	B/B	1.357	0.507	0.289
		♂ A/B	5	17	6		15	11	12			
KMY182	A	♀ A/B	A/C	A/D	B/C	B/D	A/A	A/B	B/B	16.655	0.001*	0.442
		♂ C/D	9	15	5	0	15	19	9			
	B	♀ A/B	A/A	A/B	A/C	B/C	A/A	A/B	B/B	8.613	0.035	0.474
		♂ A/C	1	12	9	9	7	18	13			
	C	♀ A/B	A/B	A/C	B/B	B/C	A/A	A/B	B/B	6.448	0.092	0.375
		♂ B/C	6	4	6	13	12	15	13			
P13F449	B	♀ A/B	A/(A or null)	A/B	B/null		A/A	A/B	B/B	2.923	0.232	0.447
		♂ A/null	12	10	4		18	17	3			
P16H420	A	♀ A/B	A/A	A/B			A/A	A/B	B/B	0.034	0.853	0.341
		♂ A/A	14	15			11	15	18			
Q64F657	C	♀ A/B	A/B	B/B			A/A	A/B	B/B	0.125	0.724	0.846
		♂ B/B	17	15			2	33	4			
H08H140	A	♀ A/B	A/C	A/D	B/C	B/D	A/A	A/B	B/B	2.571	0.463	0.317
		♂ C/D	7	10	7	4	10	13	18			
	C	♀ A/B	A/A	A/B	B/B		A/A	A/B	B/B	3.897	0.143	0.583
		♂ A/B	7	19	3		9	28	11			
HLJX-006	C	♀ A/B	A/A	A/B	B/B		A/A	A/B	B/B	2.111	0.348	0.073
		♂ A/B	4	17	6		23	3	15			
HLJX-028	C	♀ A/B	A/A	A/B	B/B		A/A	A/B	B/B	1.759	0.415	0.375
		♂ A/B	10	14	5		23	15	2			
PYMSE005	A	♀ A/B	A/C	A/D	B/C	B/D	A/A	A/B	B/B	1.129	0.770	0.548
		♂ C/D	6	10	8	7	17	23	2			
	B	♀ A/B	A/C	A/D	B/C	B/D	A/A	A/B	B/B	2.267	0.519	0.243
		♂ C/D	4	8	9	9	15	9	13			
PYER008	C	♀ A/B	A/A	A/B	A/C	B/C	A/A	A/B	B/B	14.448	0.002	0.558
		♂ A/C	11	13	5	0	5	24	14			
PYER011	C	♀ A/B	A/A	A/B	B/B		A/A	A/B	B/B	0.600	0.741	0.594
		♂ A/B	9	13	8		8	19	5			
XY105	A	♀ A/B	A/A	A/B	B/B		A/A	A/B	B/B	0.655	0.721	0.425
		♂ A/B	9	14	6		18	17	5			
	B	♀ A/B	A/B	A/C	B/B	B/C	A/A	A/B	B/B	2.515	0.473	0.500
		♂ B/C	7	8	6	12	9	19	10			
	C	♀ A/B	A/B	A/C	B/B	B/C	A/A	A/B	B/B	10.800	0.013	0.907
		♂ B/C	10	13	1	6	2	39	2			
XY129	A	♀ A/B	A/C	A/D	B/C	B/D	A/A	A/B	B/B	0.355	0.949	0.297
		♂ C/D	7	8	9	7	15	11	11			
	B	♀ A/B	A/A	A/B	A/C	B/C	A/A	A/B	B/B	4.000	0.261	0.325
		♂ A/C	4	8	12	8	9	13	18			
	C	♀ A/B	A/C	A/D	B/C	B/D	A/A	A/B	B/B	8.379	0.039	0.366
		♂ C/D	10	11	7	1	10	15	16			

(to be continued)

(continued)

Locus	Family	Parent genotype		Genotype of control progeny				Genotype of Gynogenetic progeny			χ^2	*P*	*y*
XY106	B	♀	A/B	A/C	B/C			A/A	A/B	B/B	2.133	0.144	0.205
		♂	C/C	19	11			9	8	22			
	C	♀	A/B	A/A	A/B	B/B		A/A	A/B	B/B	1.839	0.399	0.293
		♂	A/B	7	13	11		5	12	24			
FJ262375	B	♀	A/B	A/null	A/B	B/(B or null)		A/A	A/B	B/B	12.798	0.002	0.514
		♂	B/null	2	17	13		13	19	5			
	C	♀	A/B	A/(A or null)	A/B	B/null		A/A	A/B	B/B	2.733	0.255	0.727
		♂	A/null	11	8	11		2	24	7			
FJ262379	B	♀	A/B	A/null	A/B	B/(B or null)		A/A	A/B	B/B	8.613	0.013	0.314
		♂	B/null	12	1	18		7	11	17			
	C	♀	A/B	A/B	B/B			A/A	A/B	B/B	7.258	0.007	0.500
		♂	B/B	8	23			7	21	14			
FJ262380	B	♀	A/B	A/C	A/null	B/C	B/null	A/A	A/B	B/B	0.871	0.832	0.220
		♂	C/null	9	7	6	9	17	9	15			
FJ262381	A	♀	A/B	A/C	A/D	B/C	B/D	A/A	A/B	B/B	2.267	0.519	0.205
		♂	C/D	4	9	8	9	12	8	19			
	B	♀	A/B	A/A	A/B	B/B		A/A	A/B	B/B	0.613	0.736	0.122
		♂	A/B	9	16	6		17	5	19			
FJ262383	C	♀	A/B	A/B	A/C	B/B	B/C	A/A	A/B	B/B	4.500	0.212	0.103
		♂	B/C	9	11	3	9	22	4	13			
FJ262384	A	♀	A/B	A/A	A/B	A/C	B/C	A/A	A/B	B/B	15.828	0.001*	0.432
		♂	A/C	4	16	7	2	7	19	18			
	B	♀	A/B	A/C	B/C			A/A	A/B	B/B	0.000	1.000	0.179
		♂	C/C	15	15			15	7	17			
FJ262385	A	♀	A/B	A/B	A/C	B/B	B/C	A/A	A/B	B/B	8.355	0.039	0.186
		♂	B/C	8	6	14	3	12	8	23			
FJ262388	A	♀	A/B	A/A	A/B	A/C	B/C	A/A	A/B	B/B	4.250	0.236	0.184
		♂	A/C	6	13	7	6	20	7	11			
	B	♀	A/B	A/C	A/D	CB	B/D	A/A	A/B	B/B	4.667	0.198	0.273
		♂	C/D	3	11	9	7	23	12	9			
	C	♀	A/B	A/C	A/D	B/C	B/D	A/A	A/B	B/B	6.172	0.104	0.136
		♂	C/D	7	11	9	2	31	6	7			
FJ262397	C	♀	A/B	A/null	B/null			A/A	A/B	B/B	0.032	0.857	0.140
		♂	null/null	16	15			22	6	15			
FJ262401	B	♀	A/B	A/A	A/B	A/B	B/C	A/A	A/B	B/B	11.452	0.010	0.357
		♂	A/C	15	8	2	6	15	15	12			
	C	♀	A/B	A/C	A/D	B/C	B/D	A/A	A/B	B/B	12.286	0.006	0.512
		♂	C/D	13	8	7	0	12	22	9			
FJ262402	C	♀	A/B	A/A	A/B	B/B		A/A	A/B	B/B	0.500	0.779	0.545
		♂	A/B	9	14	9		8	24	12			
P3	B	♀	A/B	A/A	A/B	B/B		A/A	A/B	B/B	3.600	0.165	0.386
		♂	A/B	9	18	3		20	17	7			
P7	B	♀	A/B	A/(A or null)	A/B	B/null		A/A	A/B	B/B	2.200	0.333	0.268
		♂	A/null	11	9	10		11	11	19			
	C	♀	A/B	A/C	B/C			A/A	A/B	B/B	0.571	0.450	0.256
		♂	C/C	12	16			8	10	21			
P8	A	♀	A/B	A/B	A/C	B/B	B/C	A/A	A/B	B/B	3.750	0.290	0.475
		♂	B/C	9	12	6	5	16	19	5			
	B	♀	A/B	A/A	A/B	B/B		A/A	A/B	B/B	0.290	0.865	0.100
		♂	A/B	7	17	7		18	4	18			
	C	♀	A/B	A/A	A/B	B/B		A/A	A/B	B/B	7.867	0.020	0.250
		♂	A/B	4	12	14		17	11	16			
P11	B	♀	A/B	A/A	A/B	B/B		A/A	A/B	B/B	0.379	0.827	0.256
		♂	A/B	6	16	7		15	11	17			
	C	♀	A/B	A/A	A/B	B/B		A/A	A/B	B/B	1.414	0.493	0.628
		♂	A/B	6	13	10		2	27	14			

Notes: *P* value is from chi-square test of observed genotypic proportions compared to Mendelian expections in control progeny, *P* values with asterisks are segregation distortion at the 5% level after Bonferroni correction. *y* is estimated microsatellite-centromere recombination from the ratio of frequencies of heterozygotic genotypes in meiogynogenetic progeny.

3.3 Microsatellite-Centromere Recombination

The three scallop females used to produce gynogenetic diploids were heterozygous for 12, 19, and 22 microsatellite loci at families A, B, and C, respectively, allowing M-C distance estimation for these loci (Table 1). In most cases, similar y value was observed in the progeny from two or three gynogenetic families, thus the analysis in gynogenetic lines from different females is reproducible. In most cases, two non-recombinant homozygotes occurred in almost equal frequencies. However, the 18 genotypic ratios in gynogenetic progeny (PYMSE005, XY105, FJ262384 and P8 in family A; P13F449, FJ262379 and P3 in family B; KMY102, HLJX-028, PYER008, P7 and P11 in family C; KMY139, XY106 and FJ262388 in family B and C) deviated significantly from Mendelian expectation ($P<0.05$). This would be expected if one of the homozygotes had reduced viability because of homozygosity for the locus itself or linkage to a recessive deleterious allele.

The proportions of heterozygous offspring for the 32 loci in gynogenetic diploid families are given in Table 2. High M-C recombination frequencies were obtained at Q64F657 (0.85), XY105 (0.61) and FJ262375 (0.62), whereas low M-C recombination frequencies were observed at HLJX-006 (0.07), FJ262383 (0.10), FJ262381 (0.16) and FJ262397 (0.14). The other 26 microsatellite loci showed intermediate y values between 0.19 (FJ262385) and 0.59 (PYER011) (Table 2). The average M-C recombination frequency across loci was 0.38, corresponding to fixation index of 0.62 after a single generation of gynogenesis.

The map distances between microsatellite loci and centromeres calculated according to (1/2) y, Kosambi, and Haldane functions ranged from 4 to 42 cM, from 4 to 62 cM, and from 4 to 94 cM, respectively. When the y value was low, the map distances from the centromere were similar under the three conditions. However, with high y values, map distances were largely different (Table 2).

Table 2 Estimates of microsatellite-centromere (M-C) recombination frequencies (y), fixation indexes (F), and M-C map distances (cM) in the gynogenetic Japanese scallop

Locus	Number of Families	Number of individuals	y	F	M-C map distance (cM)		
					$x=y/2$	Kosambi	Haldane
KMY90	1	44	0.41	0.59	20	22	26
KMY102	1	46	0.57	0.43	28	32	42
KMY139	2	82	0.34±0.18[a]	0.66	17	18	21
KMY158	1	38	0.29	0.71	14	15	17
KMY182	3	121	0.43±0.05	0.57	21	23	28
P13F449	1	38	0.45	0.55	22	24	30
P16H420	1	44	0.34	0.66	17	18	21
Q64F657	1	39	0.85	0.15	42	62	94
H08H140	2	89	0.45±0.19	0.55	23	24	30
HLJX-006	1	41	0.07	0.93	4	4	4
HLJX-028	1	40	0.38	0.63	19	20	24
PYMSE005	2	79	0.40±0.22	0.60	20	21	26
PYER008	1	43	0.56	0.44	28	32	41
PYER011	1	32	0.59	0.41	30	34	45
XY105	3	121	0.61±0.30	0.39	30	35	47
XY129	3	118	0.33±0.03	0.67	16	17	20
XY106	2	80	0.25±0.06	0.75	12	13	14
FJ262375	2	70	0.62±0.15	0.38	31	36	48
FJ262379	2	77	0.41±0.13	0.59	20	22	26
FJ262380	1	41	0.22	0.78	11	11	12
FJ262381	2	80	0.16±0.06	0.84	8	8	9
FJ262383	1	39	0.10	0.90	5	5	5
FJ262384	2	83	0.31±0.18	0.69	15	16	19
FJ262385	1	43	0.19	0.81	9	9	10
FJ262388	3	126	0.20±0.07	0.80	10	10	11
FJ262397	1	43	0.14	0.86	7	7	8
FJ262401	2	85	0.43±0.11	0.57	21	23	28
FJ262402	1	44	0.55	0.45	27	31	39
P3	1	44	0.39	0.61	19	20	24
P7	2	80	0.26±0.01	0.74	13	13	15
P8	3	124	0.28±0.19	0.72	14	14	16
P11	2	86	0.44±0.26	0.56	22	24	29

Notes: y value is a mean for all families. [a] Standard deviation.

4 Discussion

It is a common phenomenon that many organisms, particularly marine invertebrates, frequently exhibit non-Mendelian segregation ratio of alleles, which can complicate the construction of a linkage map (Chu *et al*., 2014; Bai *et al*., 2015; Hollenbeck *et al*., 2015). Deviation from Mendelian segregation ratio has been observed in previous efforts of constructing molecular marker linkage maps. For microsatellite markers, distorted segregation was observed at 37.2% of loci in Pacific oyster (McGoldrick *et al*., 2000), 23.8% in sea cucumber (Li *et al*., 2009), and 11.8% in Chinese shrimp (Wang *et al*., 2008). The level of segregation distortion varies greatly among species, and likely reflects unique characteristics of different genomes. Departures from Mendelian ratio may be caused by several different factors, including selective mortality due to recessive lethal genes, sampling or genotyping errors (Launey and Hedgecock, 2001). Launey and Hedgecock (2001) demonstrated experimentally that high genetic load with resulting strong zygotic selection at the larval stage was the cause of segregation distortion in Pacific oyster. By genotyping progeny at 6 h after fertilization and then 2–3 months later, they confirmed that segregation distortion was minimal at the early zygote stage and increased during development. In this study, inheritance analysis from the control crosses showed that only two of 53 genotypic ratios (3.8%) deviated from Mendelian ratio, suggesting that segregation analysis was performed in the 70-h scallop larvae, before most defective genes were turned on and expressed.

The wide spread of null alleles at microsatellite loci is another characteristic of bivalve molluscs. Recent documents have reported high level of null alleles in marine mollusks (Li and Kijima, 2006; McGoldrick *et al*., 2000; Reece *et al*., 2004). Null alleles of microsatellite regions, which occasionally fail to yield an amplification product, may arise through mutations such as single nucleotide mutations in the primer annealing regions (Selkoe and Toonen, 2006). In this study, seven of the 32 microsatellite loci (21.8%) have null allele problems in three families. Nevertheless, they are still useful for M-C mapping when controlled crosses were performed. In population studies, the presence of null alleles may complicate the interpretation of deviations from Hardy-Weinberg equilibrium, and multiple nulls could confuse the analysis of population structure.

Gynogenetic diploids were induced with 100%, 100% and 96% success in the three gynogenetic families, respectively. There is no evidence for differential survival of the two homozygous classes in the 35 of 53 comparisons which were made at the microsatellite loci. However, at the 15 loci (KMY102, KMY139, P13F449, HLJX-028, PYMSE005, PYER008, XY105, XY106, FJ262379, FJ262384, P8, P3, P7, P11 and FJ262388) in 3 families, it was observed that the two non-recombinant homozygous genotypes were obtained unequally. Because alleles at the microsatellite locus were segregated as expected in a Mendelian ratio in the control crosses using the same female, the segregation departure might result from the induction of gynogenesis. It is possible that one of the chromosome arms in the female marked by the microsatellite locus possessed a semi-lethal or lethal allele that tightly links with the marker region. Homozygotes for this particular allelic combination probably have suffered a decreased survival rate (Li and Kijima, 2006).

Estimated M-C recombination rate varied between 0.07 and 0.85. Thus, map distance under complete interference varied between 4 and 42 cM. These results demonstrated that the microsatellite loci widely distribute from proximal (centromeric) to distal (telomeric) regions of the scallop chromosomes. Preference for an odd number of crossover (probably single) results in $y>2/3(0.67)$. This is called 'chiasma interference' (Perkins, 1955). If there is no chiasma interference, the maximum recombination rate in relation to the centromere is theoretically 2/3 (Mather, 1935). Value of $y>0.67$ shown at the single locus (Q64F657) of the 32 microsatellites (3.1%) indicates the existence of positive interference after a single chiasma formation in some scallop chromosomes. The centromere is a special domain in most important functional elements of eukaryotic chromosomes. It ensures proper cell division and stable transmission of the genetic material (Wang *et al*., 2000). Elucidating the composition and structure of centromeres can be of use to understand its functional roles, including chromosome segregation, karyotypic stability, and artificial chromosome-based cloning (Park *et al*., 2007). Identification of the genetic position of centromeres is the first step to understanding the composition and structure of the centromeric region. In this study, 32 microsatellite loci were mapped in relation to the centromere by half-tetrad analysis. The M-C distances ranged from 4 cM to 42 cM under complete interference, and would be inflated with Kosambi and Haldane's functions for larger y values. The heterogeneity of linkage groups regarding chiasma distribution pattern has been reported in some species (Nachman and Churchill, 1996; Sakamoto *et al*., 2000), which suggests that it should be cautious when applying complete interference for estimating G-C distances (Martínez *et al*., 2008).

In this study, segregation of 32 microsatellite loci was examined in normal and gynogenetic diploid families, and M-C map distances were estimated in Japanese scallop *P. yessoensis*. This is the first report of microsatellite-centromere mapping in this commercially important species. The results are important for providing fixed points in the linkage groups of genetic maps.

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