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Full Length Research Paper

A genetic linkage map of Japanese scallop *Mizuhopecten yessoensis* based on amplified fragment length polymorphism (AFLP) and microsatellite (SSR) markers

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A genetic linkage map of the Japanese scallop *Mizuhopecten yessoensis* was constructed based on 302 markers, including 263 amplified fragment length polymorphism (AFLP) markers and 39 microsatellite (SSR) markers. The two parental maps were constructed according to the double pseudo-test cross strategy with an F_1 progeny of 115 individuals. In the maternal parent, 163 markers were assigned in 20 linkage groups, spanning a total coverage of 2184.9 cM with the average spacing between two adjacent markers was 15.3 cM. In the paternal parent, 155 markers were also mapped into 20 linkage groups, spanning a genetic length of 1882.4 cM with the average marker density of 13.9 cM, respectively. The coverage estimated for the framework maps were 78.3% for the female and 77% for the male without minor linkage groups. Five full alignment linkage groups and four homologous linkage groups could be identified based on the position of 16 high information content SSRs which segregated in the parents. The construction of the *M. yessoensis* genetic linkage maps here was a part of a genetic breeding program. This linkage map will contribute to the discovery of genes, comparative genomics and quantitative trait loci in Japanese scallop.

Key words: SSR, AFLP, genetic linkage map, *Mizuhopecten yessoensis.*

INTRODUCTION

The Japanese scallop, *Mizuhopecten yessoensis*, is a cold water species which is distributed widely along the coastline of the northern islands of Japan, the northern part of the Korean Peninsula, and the Sakhalin and the Kuril Islands (Ito, 1991). It was introduced to China from Japan in 1980s. As it is larger in size and commands a higher market price than the native zhikong scallop, *Chlamys farreri,* and the introduced bay scallop, *Argopecten irradians*. The Japanese scallop aquaculture expanded rapidly and has become an important economic aquaculture species along the northern coastline of China (Xu et al., 2009). There are two types of cultivation methods: suspended culture and bottom culture. Suspended scallop cultures usually grow faster and result in a higher meat yield than bottom cultures. The bottom culturing method is based on the young scallop being released and remaining on the culture ground until harvesting and is more similar to the natural lifestyle of scallops (Maremi et al., 2005).

Due to increasing demands on both quality and quantity traits, it is necessary to continue genetic improvement of Japanese scallop with higher values. Most of the genetic improvements of the Japanese scallop, to date have been through the use of traditional selective breeding techniques, such as polyploidy breeding (Chang et al., 2002), hybridization (Yu et al., 2006), and gynogenesis (Pan et al., 2004), which have worked best on traits with additive genetic variation, but not well enough on traits with low heritability.

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Abbreviations: AFLP, Amplified fragment length polymorphism; **SSR,** microsatellite markers.

Unfortunately, many economically important traits have low heritability. Recently, molecular marker technologies, especially those based on the polymerase chain reaction (PCR), have been increasingly used in a variety of applications, including genome structure and evolution, the identification and mapping of quantitative trait loci (QTL), marker-assisted selection (MAS), positional cloning of genes and contig assembly (Chistiakov et al., 2005; Sakamoto et al., 2000; lee et al., 2005). However, genomic research and especially QTL mapping will eventually lead to marker-assisted selection (MAS) for efficient and precise selection. MAS refers to a selection process in which future breeders are chosen based on genotypes using molecular markers. To implement MAS, researchers first of all should find enough molecular markers that were linked closely with the given performance or production trait, and then determine the location of this trait on the linkage map (Sun et al., 2008).

In invertebrate, recently, some linkage maps have been reported for the zhikong scallop (Li et al., 2005;Wang et al., 2005), bay scallop (Wang et al., 2007), European flat oyster (Lallias et al., 2007a), eastern oyster (Yu and Guo, 2003), blue mussel (Lallias et al., 2007b), Pacific abalone (Liu et al., 2006), blacklip abalone (Baranski et al., 2006), and Pacific oyster (Hubert and Hedgecock, 2004; Li and Guo, 2004). Under these circumstances, the progress in genetic and genomic research on Japanese scallop has been relatively slower than other economical important marine species. There is no report on QTL analysis, but only two preliminary genetic maps. One was constructed using 56 AFLP primer combinations on 52 progeny (Liu et al., 2009); the other was published by Xu et al. (2009) based on 32 amplified fragment length polymorphism (AFLP) primer combinations and only four microsatellite (SSR) markers using 90 individuals. These previously published Japanese scallop linkage maps were built using AFLP markers or AFLP markers and few SSRs as framework that are difficult to transfer across populations or across laboratories. While these framework linkage maps are useful, the marker density is still low. Mapping additional markers, especially, appropriate anchor points, is of particular importance.

With this aim, A novel set of 26 highly polymorphic microsatellite markers, together with 13 previously developed microsatellite markers were mapped in the intervals of the fragments with AFLPs to create a more saturated and anchored linkage map for Japanese scallop. As part of the genetic breeding programs, the maps constructed in this study will be used for comparative genomics and growth discovered in the QTL analysis of Japanese scallop.

MATERIALS AND METHODS

Source of Japanese scallop Individuals of a broodstock consisting of 10 breeders from different geographic regions were genotyped with 12 polymorphic microsatellites. Genetic relationships among individuals were estimated by using genetic similarity according to Nei and Li (1979). Crosses were performed by selecting distantly related males and females to produce the largest genetic variation in the next generation. Then an inter-population hybrid pedigree of Japanese scallop was produced by crossing a field Chinese individual (male parent) and a wild Japanese individual (female parent) captured from coast of aomoriken, Japan. After 16 months suspended culture, a total of 120 progenies were randomly selected and kept in -80°C freezer.

Genomic DNA was extracted from the adductor muscle of the parents and their progenies using the standard phenol-chloroform method (Sambrook and Russell, 2001). DNA quality was evaluated on a 1% agarose gel and DNA concentration was adjusted to 2.5 µg/µL. Finally, 115 progenies were used for linkage analysis in this study. The others were abandoned because of the poor DNA quality.

Microsatellite marker analysis

A total of 39 SSRs were used to microsatellite analysis, including 26 novel microsatellite markers (Chen et al., 2010; Meng et al., 2010) and 13 previously developed primer sequences (Sato et al., 2005; Sun et al., 2007).

PCR amplification was carried out in 25 µL reaction volumes with 50 ng of template DNA, 0.4 µmol/L of each primer, 2 mmol/L MgCl2, 1×PCR Buffer (50 mmol/L KCl, 10 mmol/L Tris–HCl 0.1% Triton X-100, pH = 8.0), 200 µmol/L dNTP mixture, and 0.75 U of Taq DNA polymerase (TaKaRa, Dalian, China). Amplifications were done with an Eppendorf Mastercycler ep gradient S thermal cycler (Eppendorf, Hamburg, Germany). The PCR amplification protocol involved initial denaturing for 5 min at 94°C, followed by 26 cycles of 30 s denaturation at 94°C, annealing at the temperature given in Table 4 for 30 s, extension of 30 s at 72°C, and a prolonged extension step of 10 min at 72°C. PCR products were analysed by electrophoresis on 6% denaturing polyacrylamide gels in 1×TBE buffer and visualized with silver staining (Bassam et al. 1991). The images obtained were scanned with an Epson Perfection 2480 scanner and analysed using Gel-Pro Analyzer (version 4.5). Sizes of amplified fragments were determined by reference to a standard base-pair ladder, 20 bp DNA ladder marker (TaKaRa, Dalian, China).

AFLP analysis

AFLP analysis was carried out based on the procedures from Vos et al. (1995) with minor modifications. The digestion-ligation reaction mixture contained 3 µL genomic DNA (about 200 ng), 1. 5 µL 10×T4 DNA ligase buffer, 0.5 µL 10 mmol/L ATP, 0.1 µL of 10 mg/ml bovine serum albumin (BSA), 2.5 U MseI, 5 U EcoRI, 70 U T4 DNA ligase, 50 pmol MseI adaptor, 5 pmol EcoRI adaptor and water to bring the final volume to 12.5 µL. The reaction mixture was incubated at 37°C for 6 h. Pre-amplification was carried out using two adaptor corresponding primers each overhanging a single selective base (EcoRI adaptor primer with A and MseI adaptor primer with C). After being diluted 20-fold, the pre-amplification product was used as the template of selective amplification. Selective primers consisted of the preselective primers with three selective nucleotides added to the 3'end. EcoRI selective primers were 5' labeled with fluorescent dye TET and 6-FAM (TaKaRa, Dalian, China). The selective PCR product were scored using an ABI 3730xl DNA Analyser with GeneScan-500 (ROX) internal size standard, and results were analyzed using the software GENEMARKER 1.85 (Applied Biosystems).

Data analysis and map construction

All 39 SSR primer pairs including two expressed sequence tags

Table 1. Matrix identification of AFLP primer combinations in Japanese scallop and the mapped segregating markers (before slash), along with the total segregating markers (after slash). The skewed segregating makers were in the parenthesis.

Marker	E-AGG (A)	E-ACG (B)	E-AAC (C)	EACA (D)	E-AGC (F)	E-ACT (G)	E-AAG (H)	E-ACC (I)	E-AGA (J)	E-ATG (K)
M-CAA:1	4/8(1)				5/11(1)	3/5(0)	7/15(3)	3/7(0)	8/15(3)	4/7(0)
M-CAC:2		2/13(4)	3/9(2)	4/7(0)		6/11(2)	5/9(0)	6/11(1)	1/7(0)	
M-CAG:3	6/12(3)	2/7(0)		6/13(3)			4/9(1)	3/8(0)	6/14(3)	2/9(2)
M-CTA:4	2/6(1)	4/8(1)	7/15(3)	5/11(2)	7/14(3)	7/15(3)	7/13(2)	1/9(1)	4/9(0)	4/10(0)
M-CAT:5				5/8(1)	4/9(1)	4/9(0)		7/13(2)	2/10(0)	12/24(3)
M-CTC:6	4/11(2)	5/8(0)	9/17(4)	1/9(1)	3/7(0)	2/7(1)	5/11(1)	3/11(4)		2/9(1)
M-CTG:7	4/7(0)		3/9(2)		4/13(4)		7/14(2)	1/5(0)	5/12(2)	3/7(0)
M-CTT:8				2/8(2)	7/14(2)	8/17(5)	2/11(3)	4/11(2)	11/17(2)	6/13(2)
M-CGA:9	3/11(1)	2/7(0)		3/7(0)	4/10(0)	6/11(2)		8/17(3)		5/11(1)
M-CGT:10				4/9(1)		5/12(0)	7/13(1)	3/6(0)		
Total	323/754(103)									

AFLP, Amplified fragment length polymorphism.

(ESTs) were screened with the parental samples in order to identify the potentially polymorphic markers, and then used to genotype with 115 F1 progenies. Co-dominant SSR loci were separated into four segregation patterns: 1:1 for loci showing segregation for the female parent (type ab × aa), 1:1 for loci showing segregation for the male parent (type aa × ab), and 1:1:1:1 for loci heterozygous in both parents (type ab × cd or ab × ac) (zhang et al., 2011). Segregation data from expected 1:1:1:1-type markers into 1:1 ♀- type and 1:1 ♂-type were partitioned by creating maternal and paternal datasets to perform linkage analysis for each sex (Koshimizu et al., 2009).For AFLP markers, 100 primer combinations were used to amplify two parents and five progenies. Then 71 primer combinations that produced relatively more polymorphic fragments were used in subsequent genotyping of parents and 115 progenies. AFLP markers were scored as dominant markers: peak present (AA or Aa) or absent (aa). Only markers that showed peak present in one parent, absent in the other parent, and segregated in progeny (Aa × aa or aa × Aa) were scored for mapping analysis. AFLP markers were named after the primer pairs used to generate them and their fragment size, with EcoRI- and MseI-selective primers coded by letters and numbers, respectively (Table 1). For example, A1f100 refers to the 100 bp fragment generated by the EcoRI primer A (AGG) and the MseI primer 1 (CAA). No matter SSR or AFLP loci scored were checked for Mendelian segregation using Chi-square test for 1:1 ratios at $P < 0.05$. Markers distorted at high levels ($P < 0.05$) were discarded from the linkage analysis in order to avoid false linkage (Bert et al., 1999).

Map construction

For AFLPs, genotypes were coded as H for Aa and A for aa following the backcross model. For microsatellite where more than two alleles were involved, one of the segregating alleles was selected and coded in the same way as AFLPs in a backcross model: H for present and A for absent. The two types of markers cannot be mixed in MAPMAKER 3.0 unless codominant scoring was transformed into a dominant scoring concerning the microsatellites.

Maps were constructed according to a two-way pseudo-testcross design (Grattapaglia and Sederoff, 1994) using MAPMAKER/EXP 3.0 (Lander et al., 1987). Two linkage maps were constructed using separate data sets of markers segregating through maternal and paternal parents. Each data set was first entered in MAPMAKER as peak/fragment present “H” or absent “A”, following the F_2 backcross model. Markers were organized into linkage groups with the GROUP command at the minimum *LOD* of 3.0 and the maximum distance between two loci of 40 cM. For groups with nine or less markers, the most likely order within each group was determined using the COMPARE command.

For groups with more than nine markers, the THREE POINT and ORDER commands were used to obtain the order of markers with unique placement, followed by the TRY command to find the most likely placement of the remaining markers. The final order of each group was tested with the RIPPLE function. Additional markers that could not be mapped with the previous commands were incorporated using the NEAR and TRY commands. Typing errors were detected with the ERROR DETECTION and TRIPLE ERROR DETECTION command (Lincoln and Lander, 1992), and the map distances in centiMorgan (cM) were calculated in Kosambi’s mapping function (Kosambi, 1944). Maps were drawn using MapChart software 2.1 (Voorrips, 2002).

Genome length and coverage

Both map length and map coverage calculated based on framework markers. Average framework marker spacing (*s*) was calculated by dividing the sum length of all linkage groups by the number of intervals. Two approaches were used to estimate the genome length. (1) G_{e1}: The genome was estimated by simply adding 2*s* to the length of every group to account for the two chromosome ends beyond the terminal of each linkage group (Fishman et al., 2001); (2)

G_{e2}: According to method 4 of (Chakravarti et al., 1991), Each linkage group was multiplied by the factor (m+1)/(m-1), where m is the marker number on each linkage group. The estimated genome length is the sum of revised length of all linkage groups. The average of the two estimates was used as the estimated genome length (G_e) for the Japanese scallop. Two observed genome lengths were calculated, one as the length of the framework map (G_{of}), and the other as the total length considering all markers (G_{oa}) (Cervera et al., 2001), and considering all markers on the framework map, the triplets and the doublets. The observed genome coverages, C_{of} and C_{oa}, were determined by G_{of}/G_e and C_{oa}/G_e respectively.

RESULTS

Molecular markers

All tested microsatellite markers were polymorphic in two parental trees. In the mapping population, 23 were intercross configuration (12 in female and 11 in male) and 16 segregated in both parents. No significant deviation from the expected Mendelian ratio was observed.

In AFLP analysis, a total of 71 primer combinations were used for the map construction. 754 peaks were polymorphic out of 2774 detected peaks, accounting for 27.2% of the total. Each primer combination produced 24 to 75 peaks with the size between 50 to 500 bp. The frequency of polymorphic markers per primer combination ranged from 16.2 to 42.9% (Table 1). Of the 754 polymorphic markers detected, 426 segregated in a 1:1 ratio. Among the 426 markers, 220 were segregating through the female parent, and 206 through the male parent. Chi-square analysis indicated that 323 (167 in females and 156 in males) gave a compatible fit to the expected 1:1 segregation ratio, and 103 (53 in females and 50 in males) showed significant segregation distortion ($P < 0.05$).

Linkage map

A total of 362 (39SSRs + 323 AFLPs) markers were used to build the parental maps. For the female map, 163 of the195 maternal markers (28 SSRs + 135 AFLPs) were assigned to 20 linkage groups (more than three markers; Figure 1) covering 2184.9 cM (on average 109.2 cM per group and 8.2 markers per group). The marker spacing ranged between 8.4 and 19.4 cM for each group, with an average of 15.3 cM. 32 markers were not linked to the linkage groups, including two triplets, four doublets and 18 unlinked markers. For the male map,155 of the183 paternal markers (27 SSRs + 128 AFLPs) were also mapped to 20 linkage groups (Figure 1) covering 1882.4 cM (on average 94.1 cM per group and 7.8 markers per group). The marker spacing on each group varied between 7.6 and 23.8 cM with an average of 13.9 cM. Two triplets and three doublets were obtained while 16 single markers were unlinked.

All 3 9 SSRs (including 2ESTs) used herein were successfully mapped onto the parent maps. Of the 39 loci analyzed here, 28 (72%) SSR loci were heterozygousand segregated in the female parent and mapped to 16 groups. 27 (69%) SSR loci were mapped in the male parent to 13 groups. 16 (41%) SSR loci (FJ262384, EF056536, FJ262384, EF056526, GQ167165, FJ262409, GQ167156, DQ679222, FJ262399, EF056534, FJ262372, DQ221720, AB194038, FJ262374, FJ262401 and GT087672) segregated in both parents in a fully informative fashion with three or four different alleles. Based on the position of these 16 SSRs which segregated in both parents, five full alignment linkage groups (at least two common SSR markers) and four homologous linkage groups (only one common SSR marker) could be identified (Figure 1). In addition, The Shapiro–Wilk test demonstrated that markers among different chromosomes for both the female and male maps are randomly distributed ($P > 0.05$, after Bonferroni correction) (Table 2).

Genome length and coverage

Based on framework markers, map lengths were estimated to be 2796.1 cM (G_{e1}) and 2782.8 cM (G_{e2}) using two methods with the average of 2789.4 cM (G_e) for the female. In the male, the estimated map length was 2440 cM and 2448.1 cM with the average of 2444 cM. Based on the observed length of the framework map (2184.9 cM and 1882.4 cM) and the estimated map length (2789.4 cM and 2444 cM), genome coverage of male and female framework maps was 78.3 and 77%, respectively. Considering all the triplets and doublets, the map coverage increased to 82.3% for the female and 80.9% for the male (Table 3).

Male and female linkage groups are indicated with “F” and “M”. Filled bars show the male linkage groups and the open bars show the female linkage groups, respectively. Genetic distances are given in cM on the left side of the linkage groups, and markers are listed on the right side of the linkage groups. Microsatellite markers are underlined. 16 bridge markers are interconnected with dotted lines.

DISCUSSION

Mapping family and experimental design

The maps were created by using a two-way pseudo-testcross mapping strategy (Gratapaglia and Sederoff, 1994). The pseudo-testcross strategy is used for the construction of genetic linkage maps of highly heterozygous organisms. In this study, 10 breeders from different geographic regions were used to genotype with twelve polymorphic microsatellites. Genetic relationships among individuals were calculated by using genetic similarity according to Nei and Li (1979). Pedigree hybrids were

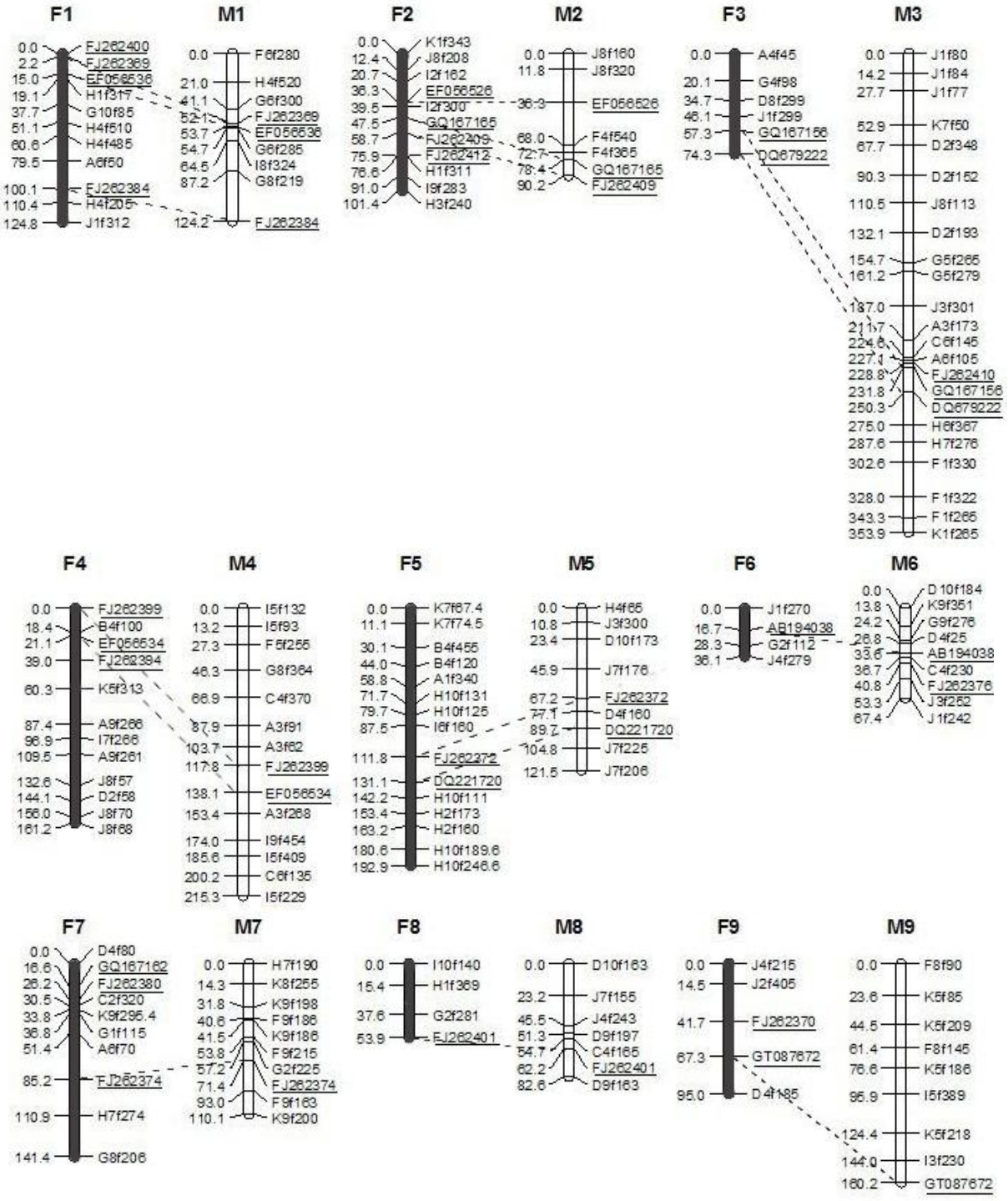

Figure 1. Japanese scallop linkage maps showing AFLP and SSR markers. AFLP, Amplified fragment length polymorphism; SSR, microsatellite markers.

obtained by crossing selecting distantly related Chinese individual (male parent) and a Japanese individual (female parent). Our experimental design has been successfully used to ensure high levels polymorphism of hybrids pedigree of Japanese scallop for the construction of linkage genetic maps.

Genetic linkage map

In the current maps, the novel set of 26 highly polymorphic microsatellite markers and 13 previously developed microsatellite markers were mapped in the intervals of the fragments with AFLPs to create a more saturated

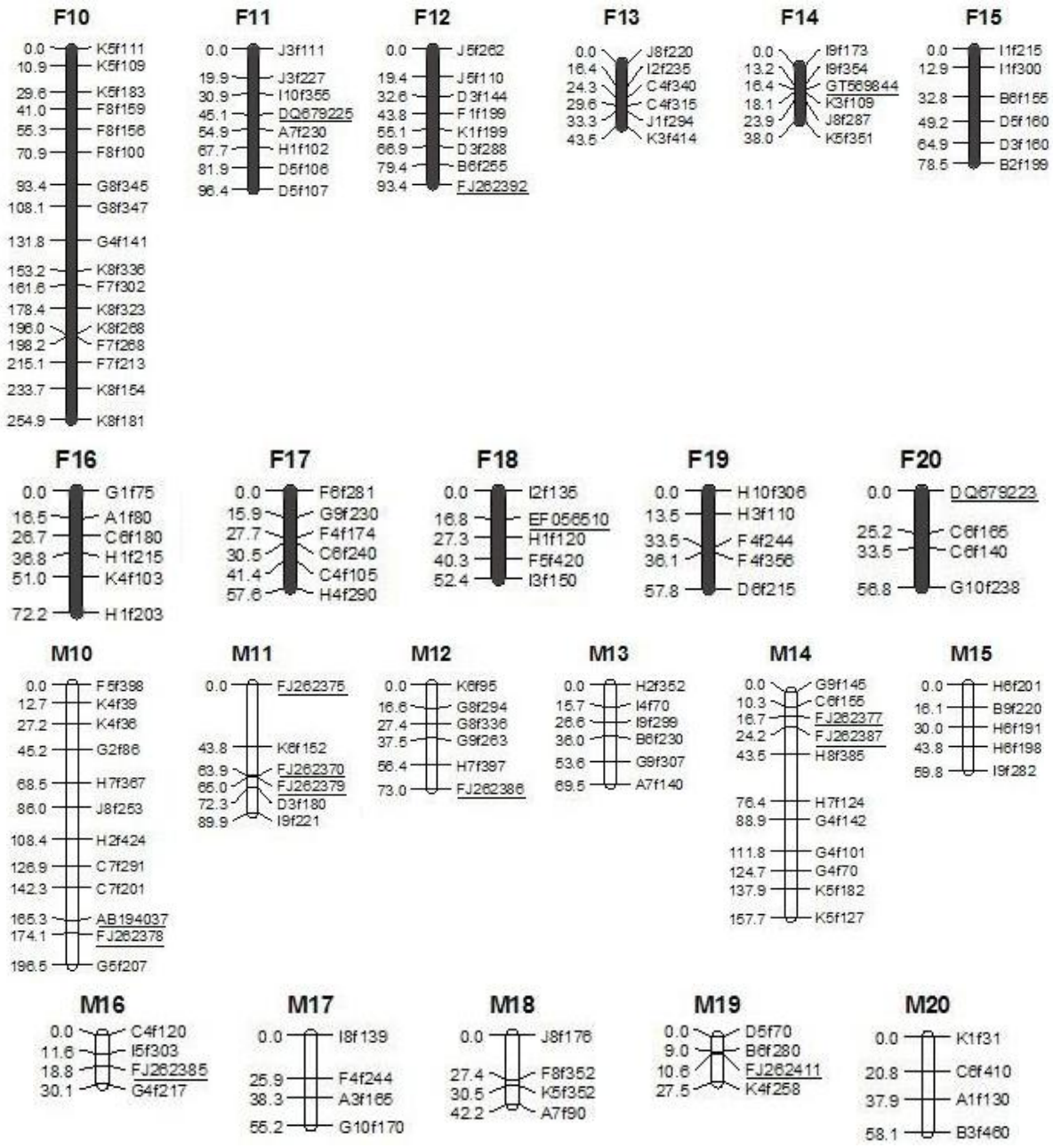

Figure 1. Contd.

and anchored linkage map for Japanese scallop and we used more F_1 progeny to avoid the unobservable phenomenon of linkage exchange which resulted from low sample number. Theoretically, at least two SSR loci are needed for a full alignment of linkage groups. For linkage groups carrying only one SSR marker, the orientation of the group remains unclear. In this study, based on the position of 16 SSRs which segregated in the parents, five full alignment linkage groups and four homologous linkage groups could be identified. Unfortunately, the genome size of consensus map is still short because of the limited number of mapped SSR markers. A higher number of SSR markers on the maps still would be desirable.

The current constructed genetic linkage maps of Japanese scallop consisted of 20 linkage groups both for female and male. This result was little different from the previously constructed genetic linkage maps of Japanese scallop. The linkage maps constructed by Liu et al. (2009) gained 22 groups for both the maternal and parental maps (including triplets), and the maps constructed by Xu et al. (2009) produced 20 linkage groups

Table 2. Summary statistics for the female and male maps of Japanese scallop.

Feature	Female	Male
AFLP markers used	167	156
Microsatellites used	28	27
Total markers used	195	183
Framework markers	163	155
Unlinked triplets	2	2
Unlinked doublets	4	3
Unlinked singles	18	16
Linkage groups (≥4 markers)	20	20
Maximum number of markers per group	23	17
Minimum number of markers per group	4	4
Average number of markers per group	8.15	7.75
Maximum average interval (cM)	19.4	23.8
Minimum average interval (cM)	8.4	7.6
Average marker spacing (cM)	15.3	13.9
Maximum length of linkage group (cM)	353.9	254.9
Minimum length of linkage group (cM)	27.5	36.1
Average length of linkage group (cM)	109.2	94.1
Total length of framework map (cM)	2184.9	1882.4

AFLP, Amplified fragment length polymorphism.

Table 3. Map length and genome coverage for Japanese scallop.

Map lenght	Values	
Observed length (cM)		
Gof	2184.9	1882.4
Goa	2296.2	1978.1
Estimated length (cM)		
Ge1	2796.1	2440
Ge2	2782.8	2448.1
Average Ge	2789.4	2444
Genome coverage (%)		
Cof	78.3	77
Coa	82.3	80.9

for female and 21 linkage groups for male, respectively.

Genetic linkage maps are indirect measures of the number of physical chromosomes. When sufficient coverage is achieved, the numbers of linkage groups should be consistent with the haploid chromosome number of the genome. The Japanese scallop is a typical diploid marine organism. The number of linkage groups reported here is slight higher than the haploid chromosome number of Japanese scallop (n=19) (Komaru and Wada, 1985), indicating that some linkage groups belong to the same chromosomes.

Map length genome length and coverage

In this study, the length of the genetic map for the female parent was estimated to be 2184.9 cM and that of the male parent to be 1882.4 cM, showing obvious differences in different sexes. In human, mouse, cattle, pig, fish and, indeed, most animals studied so far, Female maps are usually longer than male maps. This is also similar to the situation reported from various mollusks species including the pacific oyster (Li and Guo, 2004), zhikong scallop (Wang et al., 2005) and European

Table 4. Sequence and amplified result of microsatellite loci.

GenBank No.	Forward sequence	Reverse sequence	Motif	*Tm*
FJ262400	TCAGTGTGATAAAGCCAGACTCA	TGAGGATGATACTTTGGTGATTG	$(ATC)_{15}$	60
FJ262369	TCAGTGTGATAAAGCCAGACTCA	TGAGGATGATACTTTGGTGATTG	$(TCA)_{27}$	62
FJ262384	TCTGGATTGTCATTGTGAAGATG	TGACGGAATAATCTCCCTTGA	$(TGA)_{6}$	54
FJ262412	CCCAAAAAGAACCGTCATGT	ATAGACGGAGGGACGGATTT	$(GAT)_{14}$	60
FJ262410	AATGATTGCTGCTGCTGTTG	TCGACGATCAGACAAAGACACT	$(ATG)_{6}$	60
FJ262409	CGACGATCACTGTAATTCAACA	CGACGATCACTGTAATTCAACA	$(TCA)_{16}$	60
FJ262399	CAAGAGCACAATCGACCTCA	TCATCATCGTCGTCGTTGTC	$(TGA)_{7}$	60
FJ262394	CCTCATCTTCGGATTCATCA	CGATCAAGATGGCACTCAAA	$(TCA)_{13}$	60
FJ262372	TCGACGATCAACCAGAAATG	CGATCAATGGATTGAATGATGA	$(CAT)_{11}$	52
FJ262376	CGATCTAGGAGCGTGTGTCC	CAATCAGGATGATGTTGACGA	$(CAT)_{9}$	60
FJ262380	TGTTGTGAGGGCAAGATTCA	GACGATCCCTACGACCTTGA	$(GT)_{16}$	60
FJ262374	ATCCAGGCAACCGTTGATAC	CGACGATCATGTATTTCTGGTG	$(TCA)_{9}$	60
FJ262401	CCCTTTCTTGCCAAGTCTTTT	AATGGCAAATTGATGATGATG	$(ATC)_{9}$	60
FJ262370	TCGACGATCAAAGCACATAA	TAAGACGACGAAGACGACGA	$(TCA)_{9}$	54
FJ262378	TTCATGGACAGATTCAGTGTGA	TGAGGATGATACTTTGGTGATTG	$(CAT)_{6}$	60
FJ262375	TCGACGATCAACAAGACATATT	GTGTTCCTGTAGGCCTCGAA	$(TGA)_{8}$	58
FJ262370	TCGACGATCAAAGCACATAA	TAAGACGACGAAGACGACGA	$(TCA)_{9}$	54
FJ262379	GTGTTCCTGTAGGCCTCGAA	TCGACGATCAACAAGACATATT	$(ATC)_{8}$	58
FJ262392	GGAATGCTGAAAGTTTATTGTTGA	CGACGATCACATCATAACACTCC	$(TGA)_{14}$	58
FJ262386	TCGACGATCATCCTCAACAT	CCTTTTGCAGTCAACTTTTCTTT	$(TCA)_{22}$	54
FJ262377	CGATCAAGATGGCACTCAAA	TTCCCAAGCAGACATCCTTC	$(CAT)_{19}$	60
FJ262387	CGATCAAGATGGCACTCAAA	TTCCCAAGCAGACATCCTTC	$(GAT)_{19}$	58
FJ262385	TGTAAAGGCGCAAATGACTG	TGCACCACCTCACCAAAATA	$(ATG)_{9}$	58
FJ262411	CATGGTTTGCGAATTTGTTG	GTTATTTTGGGGCGGTTTTC	$(TGA)_{17}$	60
GT087672	GTCAGGGCCATCGATAAAGA	TCTCTGTCGATCCCTTTTGG	$(TGA)_{8}$	60
GT569844	TGCGAATTAAAGGTTTGATGG	AACGACAGACAGACCACTGAG	$(TA)_{7}$	60
EF056536	TTTGGCAGACAACATTTTTCA	CAATCAACAAAATCAGGAACCA	$(GA)_{14}$	58
EF056526	TCGTGTGACATTTTGATTATGCT	GCAGACCAGACATGAATGAATC	$(AG)_{17}$	60
GQ167165	TCCAAGAGAGTGTGGGCTTT	AGTCTTCAGGGGTGAACGAG	$(GT)_{7}$	60
GQ167156	ACCTCTTGTCAAAAAGGATGAG	ACAACCACATGGTCCGAAAT	$(AG)_{27}$	54
DQ679222	GGCGAGATGGCCATATTTTA	TGAGGGAGACCTTTTCGTTG	$(AG)_{28}$	60
EF056534	TGCACCACACTCTCACTCAG	CCGCAAAATTGTGTGTATGG	$(AG)_{9}$	58
DQ221720	ATGCGGAGATTGAAACCAAG	AGGACGGTAGAGCTGAGTCG	$(GA)_{13}$	60
AB194038	TTCGAATGCAAAGAAAAGCA	CCAGTTATTGCTCAGTTTAATGAAT	$(TG)_{15}$	56
GQ167162	TGAAATCAACGCCAATGAAA	GGGGTAAGCATACGTGGCTA	$(GA)_{8}$	54
AB194037	TTGTGTAGTGCACTGTAGTTTGG	ACACCGATCCCATTTCTCAA	$(TTGA)_{8}$	56
DQ679225	GTGCAGAGAGCGCGTATGT	CCTCGACCCAAAGTCCATAA	$(GA)_{30}$	62
EF056510	GTTTATTGTACGCGGCGTCT	CTGCAGGTCGACGATTAGTG	$(AG)_{13}$	60
DQ679223	TGGTAGCAGATGTTTCCCTGT	GAGCAGGGAGGCGATTATCT	$(GA)_{21}$	58

flat oyster (Lallias et al., 2007a) etc. The molecular mechanism responsible for the differences in recombination rates between the two sexes is not currently well understood. In this study, as other species, the female map was longer than the male map, although we cannot localize differences in the recombination rates to centromeres or telomeres (Zhang et al., 2007). Differences in map length can result from a variation in the number of recombination events in the two parents as well as variations in the numbers and locations of mapped loci. Thus, differences in observed and estimated genome lengths can only be seen as indicators of potential sex-related differences in recombination rates. Recombination differences between the sexes can only be assessed accurately by pairwise comparisons between pairs of anchor loci mapped in the two parental maps.

Despite the presence of gaps, the maps constructed in this study provide reasonably good coverage of the Japanese scallop genomes, 82.3% for the female map

and 80.9%for the male map when including the triplets and doublets. Compared with the two previous *M. yessoensis* maps, 76.8% for the maternal map and 79.3% for parental map (Liu et al., 2009), and 78.2% for the female map and 79.3% for the male map (Xu et al., 2009), the genetic linkage maps presented are improved. One EST Library and a transcriptome sequencing of Japanese scallop are under construction in our laboratory, and more genetic microsatellite derived from EST sequences and single nucleotide polymorphism (SNP) markers will be developed in the future. With the development of codominant markers for Japanese scallop, they will increase the genome coverage, construct high-resolution linkage maps, and finally make a high density common linkage map cross female and male.

Conclusion

Genetic linkage maps play a prominent role in many areas of genetics, including QTL analysis, map-based gene cloning, MAS, and comparative genomics. The current study was developed as a tool for comparative genomics, genetic improvement research to identify QTLs connected with economically important traits, and to promote the progress of genetic breeding programs in Japanese scallop.

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