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A first-generation linkage map of the Pacific lion-paw scallop (*Nodipecten subnodosus*): Initial evidence of QTL for size traits and markers linked to orange shell color

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ABSTRACT

The first genetic linkage maps of the Pacific lion-paw scallop were created using microsatellite and AFLP genotyping of a full-sibling family spawned from a single, pairwise mating. As an important step in the development of genomic resources for this species, the maps were then used in an effort to identify putative quantitative trait loci (QTL) for the size traits of shell length, height, and width, as well as total mass, soft tissue mass, muscle mass, and orange shell color. The female map contains 147 markers on 27 linkage groups, covering 919.7 cM, while the male map contains 149 markers placed on 20 linkage groups spanning 963.7 cM. Combining all available markers resulted in a consensus map of 320 loci (56 microsatellites and 264 AFLPs) on 22 linkage groups with an average spacing of 3.8 cM and a total map distance of 1124.14 cM. Non-parametric, Kruskal–Wallis tests found significant linkage at $\alpha = 0.005$ for two size traits: total biomass in the loci segregating in the female parent, and shell width in the male. Interval mapping showed chromosome-wide significance for many traits on two primary linkage groups in the female map and one in the male. Highly correlated, 91.7% of the variation among the six size phenotypes could be explained by the first principal component, which explained 9 and 7.8% of the variance in the female and male maps, respectively. With the exception of muscle mass mapped when maturation stage was considered as a covariate, no significant LOD scores for size traits were identified using the combined map. Finally, orange shell color was mapped to a 1 cM region of linkage group Nsub9. The development of these maps and identification of linkage groups linked to size traits provide a foundation for future quantitative studies, which could ultimately result in marker assisted selection to improve scallop growth in aquaculture.

1. Introduction

While model organisms and many species significant to agriculture have been subject to full genome sequencing, the genomic resources for organisms such as the Pacific lion-paw scallop (*Nodipecten subnodosus*) are few. With a significant economic value, the large and fast-growing Pacific lion-paw scallop is of interest to aquaculture ventures. The development of microsatellite markers (Ibarra et al., 2006; Petersen et al., 2009) has allowed for population-level investigations of diversity in aquaculture (Petersen et al., 2008) and the wild (Petersen et al., 2010), but still little information exists regarding the organization of the genome. The creation of a genetic linkage map is a common first step in the development of genetic resources for non-model organisms. Linkage maps specific for this species will allow for the identification of genetic markers associated with heritable traits important to aquaculture. Traits of interest include those involved in growth and size, particularly of the adductor muscle, which is marketed for human consumption. While not quantified in the Pacific lion-paw scallop, the heritability of such size traits have been investigated in other species of scallop (Crenshaw et al., 1991; Ibarra et al., 1999; Liang et al., 2010; Perez and Alfonsi, 1999; Zheng et al., 2004); the results from the aforementioned studies as well as observed variation within and between families of *N. subnodosus* spawned in the laboratory suggests that these traits have a genetic basis in the Pacific lion-paw scallop as well. As each individual scallop can spawn as many as 25 million eggs per spawning event (Maldonado-Amparo et al., 2004), the identification of even a few superior spawners could result in a significant increase in the size of the population. If QTL for growth traits can be identified, a selective breeding program can utilize those loci to increase aquaculture production, which would benefit producers and potentially reduce harvest pressure on declining natural stocks.

Several mollusks have already been the focus of genetic mapping using microsatellite and/or AFLP markers. These include the blue mussel (Lallias et al., 2007), Pacific oyster (Hubert and Hedgecock,

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Table 1
Linkage group information for (a) combined, (b) female, and (c) male maps. Female and male linkage groups are listed next to the combined linkage group to which they correspond based upon microsatellite placement. Length and distance measurements are given in centiMorgans.

(a)

Linkage group	Total markers	Micro-satellites	AFLP loci	Total length (cM)	Avg spacing	Corresponding LG	
						Maternal	Paternal
Nsub1	15	5	10	135.41	9.67	15, 19, 26	19, 20
Nsub2	28	5	23	99.48	3.68	3, 8	2, 9
Nsub3	26	7	19	93.33	3.73	2	12
Nsub4	17	4	13	74.25	4.64	5	6
Nsub5	16	1	15	71.28	4.75	11	1
Nsub6	20	3	17	69.50	3.66	1	8
Nsub7	20	2	18	68.09	3.58	4, 12	14
Nsub8	10	1	9	65.98	7.33	6	4
Nsub9	24	4	20	59.56	2.59	14, 24	3
Nsub10	21	1	20	57.58	2.88	10, 23	13
Nsub11	16	3	13	55.68	3.71	16, 21	10
Nsub12	20	5	15	51.54	2.71	1	7
Nsub13	19	3	16	48.81	2.71	9	16
Nsub14	16	2	14	47.08	3.14	7	5
Nsub15	20	5	15	35.59	1.87	22	11
Nsub16	8	1	7	29.33	4.19	18	18
Nsub17	5	0	5	18.95	4.74	13*	n/a
Nsub18	6	1	5	18.92	3.78	20	15,16
Nsub19	3	0	3	12.08	6.04	17*	n/a
Nsub20	4	2	2	6.37	2.12	6	n/a
Nsub21	3	0	3	4.09	2.05	27[a]	n/a
Nsub22	3	1	2	1.24	0.62	25	17
Average	14.55	2.55	12.00	51.10	3.83		
Total	320	56	264	1124.14	84.21		

(b)

Linkage group	Total markers	Micro-satellites	AFLP loci	Total length (cM)	Avg spacing
NsubF1	20	7	13	182.56	9.61
NsubF2	14	6	8	96.76	7.44
NsubF3	11	4	7	74.74	7.47
NsubF4	6	1	5	74.68	14.94
NsubF5	8	4	4	68.37	9.77
NsubF6	7	3	4	59.14	9.86
NsubF7	6	2	4	55.57	11.11
NsubF8	3	0	3	48.29	24.14
NsubF9	4	2	2	41.29	13.76
NsubF10	4	0	4	31.00	10.33
NsubF11	5	1	4	22.20	5.55
NsubF12	5	1	4	20.53	5.13
NsubF13	4	0	4	19.84	6.61
NsubF14	8	3	5	18.95	2.71
NsubF15	2	2	0	16.59	16.59
NsubF16	4	1	3	13.68	4.56
NsubF17	3	0	3	12.08	6.04
NsubF18	4	1	3	11.98	3.99
NsubF19	2	2	0	11.90	11.90
NsubF20	2	1	1	11.90	11.90
NsubF21	4	2	2	10.48	3.49
NsubF22	8	4	4	9.59	1.37
NsubF23	4	1	3	3.84	1.28
NsubF24	2	0	2	2.53	2.53
NsubF25	3	1	2	1.24	0.62
NsubF26	2	1	1	0.00	0.00
NsubF27	2	0	2	0.00	0.00
Average	5.44	1.85	3.59	34.06	7.51
Total	147	50	97	919.71	202.72

(c)

Linkage group	Total markers	Micro-satellites	AFLP loci	Total length (cM)	Avg spacing
NsubM1	8	1	7	101.11	14.44
NsubM2	5	0	5	67.87	16.97
NsubM3	12	4	8	65.48	5.95
NsubM4	6	1	5	64.38	12.88
NsubM5	9	2	7	59.89	7.49
NsubM6	9	4	5	58.47	7.31
NsubM7	13	3	10	57.98	4.83
NsubM8	7	2	5	52.20	8.70
NsubM9	7	4	3	50.89	8.48
NsubM10	8	3	5	50.64	7.23
NsubM11	7	3	4	44.56	7.43
NsubM12	11	6	5	44.50	4.45
NsubM13	9	1	8	41.26	5.16
NsubM14	7	2	5	39.98	6.66
NsubM15	4	1	3	39.10	13.03
NsubM16	9	3	6	38.40	4.80
NsubM17	5	1	4	34.56	7.89
NsubM18	3	1	2	28.19	14.09
NsubM19	8	3	5	27.20	3.89
NsubM20	2	2	0	0.00	0.00
	7.45	2.35	5.10	48.18	8.08
	149	47	102	963.66	161.68

[a] Based exclusively upon AFLP loci.

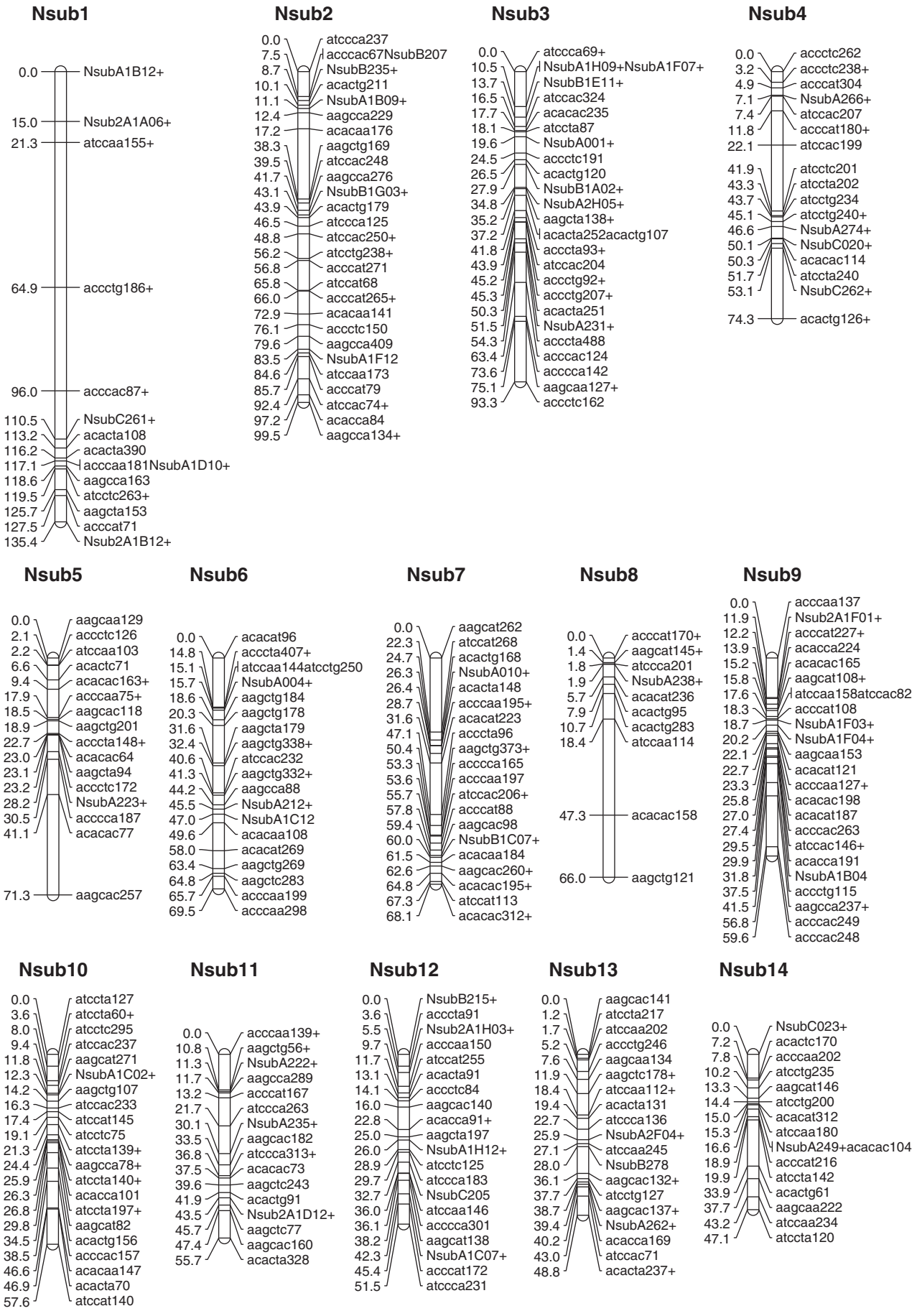
2004; Li and Guo, 2004), eastern oyster (Yu and Guo, 2003), Zhikong scallop (Wang et al., 2004, 2005), and abalone (Baranski et al., 2006; Sekino and Hara, 2007; Shi et al., 2010; Zhan et al., 2011). Some investigations have also mapped loci associated with sex (Li et al., 2005), disease resistance (Yu and Guo, 2006), size (Qin et al., 2007a; Zhan et al., 2009), and shell color (Qin et al., 2007b; Zhan et al., 2009). In mollusk aquaculture, selective breeding has been successful in several species, most often with the goal of achieving larger size and/or faster growth (Deng et al., 2009; He et al., 2008; Ibarra et al., 1999; Langdon et al., 2003; Zheng et al., 2004), or to increase a population's resistance to disease (Nell and Perkins, 2003; Ragone Calvo et al., 2003). However, marker assisted selection in mollusk aquaculture is thus far uncommon due largely to the limited availability of genomic resources.

The purpose of the current study was to construct the first genetic map of the Pacific lion-paw scallop using a full-sibling family. Seventy-two previously published microsatellite markers (Ibarra et al., 2006; Petersen et al., 2009) and one new microsatellite locus were available to anchor the map, while AFLP loci were used to link microsatellites and facilitate more dense coverage of the genome. Although the Pacific lion-paw scallop is a functional hermaphrodite, the separate collection of sperm and eggs from two different individuals allowed for a single-pairwise mating and thus the development of a "male," "female," and combined linkage map. These first-generation maps were then used in an attempt to identify markers linked to growth traits and orange shell color. Adding data from additional families and markers will allow for the identification of chromosome regions, individual genes, and ultimately polymorphisms that affect size in this species.

2. Materials and methods

2.1. Spawning and culture

Wild spawners from the Lagoon Ojo de Liebre, Mexico were obtained in July 2007, and transported to the laboratory with aeration at 17 °C. Once back in the Aquaculture Genetics and Breeding Laboratory (CIBNOR, La Paz, Mexico), the scallop utilized as the female parent was induced to release gametes via temperature shock, increasing the temperature to 24 °C. Because sperm is released prior to the eggs in these functional hermaphrodites, the scallop acting as the female parent was moved into a new container after sperm was released to collect only eggs. To prevent self-fertilization of the eggs by any sperm remaining in the scallop, the first eggs spawned were discarded. In a separate container the scallop that would become the male parent was induced to spawn through the injection of 0.4 ml of serotonin (0.5 mM) into the adductor muscle. Eggs from the former


Nsub1
0.0 NsubA1B12+
15.0 Nsub2A1A06+
21.3 atccaa155+
64.9 accctg186+
96.0 acccac87+
110.5 NsubC261+
113.2 acacta108
116.2 acacta390
117.1 acccaa181NsubA1D10+
118.6 aagcca163
119.5 atcctc263+
125.7 aagcta153
127.5 acccat71
135.4 Nsub2A1B12+
Nsub2
0.0 atccca237
7.5 acccac67NsubB207
8.7 NsubB235+
10.1 acactg211
11.1 NsubA1B09+
12.4 aagcca229
17.2 acacaa176
38.3 aagctg169
39.5 atccac248
41.7 aagcca276
43.1 NsubB1G03+
43.9 acactg179
46.5 atccca125
48.8 atccac250+
56.2 atcctg238+
56.8 acccat271
65.8 atccat68
66.0 acccat265+
72.9 acacaa141
76.1 accctc150
79.6 aagcca409
83.5 NsubA1F12
84.6 atccaa173
85.7 acccat79
92.4 atccac74+
97.2 acacca84
99.5 aagcca134+
Nsub3
0.0 atccca69+
10.5 NsubA1H09+NsubA1F07+
13.7 NsubB1E11+
16.5 atccac324
17.7 acacac235
18.1 atccta87
19.6 NsubA001+
24.5 accctc191
26.5 acactg120
27.9 NsubB1A02+
34.8 NsubA2H05+
35.2 aagcta138+
37.2 acacta252acactg107
41.8 acccta93+
43.9 atccac204
45.2 accctg92+
45.3 accctg207+
50.3 acacta251
51.5 NsubA231+
54.3 acccta488
63.4 acccac124
73.6 acccca142
75.1 aagcaa127+
93.3 accctc162
Nsub4
0.0 accctc262
3.2 accctc238+
4.9 acccat304
7.1 NsubA266+
7.4 atccac207
11.8 acccat180+
22.1 atccac199
41.9 atcctc201
43.3 atccta202
43.7 atcctg234
45.1 atcctg240+
46.6 NsubA274+
50.1 NsubC020+
50.3 acacac114
51.7 atccta240
53.1 NsubC262+
74.3 acactg126+
Nsub5
0.0 aagcaa129
2.1 accctc126
2.2 atccaa103
6.6 acactc71
9.4 acacac163+
17.9 acccaa75+
18.5 aagcac118
18.9 aagctg201
22.7 acccta148+
23.0 acacac64
23.1 aagcta94
23.2 accctc172
28.2 NsubA223+
30.5 acccca187
41.1 acacac77
71.3 aagcac257
Nsub6
0.0 acacat96
14.8 acccta407+
15.1 atccaa144atcctg250
15.7 NsubA004+
18.6 aagctg184
20.3 aagctg178
31.6 aagcta179
32.4 aagctg338+
40.6 atccac232
41.3 aagctg332+
44.2 aagcca88
45.5 NsubA212+
47.0 NsubA1C12
49.6 acacaa108
58.0 acacat269
63.4 aagctg269
64.8 aagctc283
65.7 acccaa199
69.5 acccaa298
Nsub7
0.0 aagcat262
22.3 atccat268
24.7 acactg168
26.3 NsubA010+
26.4 acacta148
28.7 acccaa195+
31.6 acacat223
47.1 acccta96
50.4 aagctg373+
53.3 acccca165
53.6 acccaa197
55.7 atccac206+
57.8 acccat88
59.4 aagcac98
60.0 NsubB1C07+
61.5 acacaa184
62.6 aagcac260+
64.8 acacac195+
67.3 atccat113
68.1 acacac312+
Nsub8
0.0 acccat170+
1.4 aagcat145+
1.8 atccca201
1.9 NsubA238+
5.7 acacat236
7.9 acactg95
10.7 acactg283
18.4 atccaa114
47.3 acacac158
66.0 aagctg121
Nsub9
0.0 acccaa137
11.9 Nsub2A1F01+
12.2 acccat227+
13.9 acacca224
15.2 acacac165
15.8 aagcat108+
17.6 atccaa158atccac82
18.3 acccat108
18.7 NsubA1F03+
20.2 NsubA1F04+
22.1 aagcaa153
22.7 acacat121
23.3 acccaa127+
25.8 acacac198
27.0 acacat187
27.4 acccac263
29.5 atccac146+
29.9 acacca191
31.8 NsubA1B04
37.5 accctg115
41.5 aagcca237+
56.8 acccac249
59.6 acccac248
Nsub10
0.0 atccta127
3.6 atccta60+
8.0 atcctc295
9.4 atccac237
11.8 aagcat271
12.3 NsubA1C02+
14.2 aagctg107
16.3 atccac233
17.4 atccat145
19.1 atcctc75
21.3 atccta139+
24.4 aagcca78+
25.9 atccta140+
26.3 acacca101
26.8 atccta197+
29.8 aagcat82
34.5 acactg156
38.5 acccac157
46.6 acacaa147
46.9 acacta70
57.6 atccat140
Nsub11
0.0 acccaa139+
10.8 aagctg56+
11.3 NsubA222+
11.7 aagcca289
13.2 acccat167
21.7 atccca263
30.1 NsubA235+
33.5 aagcac182
36.8 atccca313+
37.5 acacac73
39.6 aagctc243
41.9 acactg91
43.5 Nsub2A1D12+
45.7 aagctc77
47.4 aagcac160
55.7 acacta328
Nsub12
0.0 NsubB215+
3.6 acccta91
5.5 Nsub2A1H03+
9.7 acccaa150
11.7 atccat255
13.1 acacta91
14.1 accctc84
16.0 aagcac140
22.8 acacca91+
25.0 aagcta197
26.0 NsubA1H12+
28.9 atcctc125
29.7 atccca183
32.7 NsubC205
36.0 atccaa146
36.1 acccca301
38.2 aagcat138
42.3 NsubA1C07+
45.4 acccat172
51.5 atccca231
Nsub13
0.0 aagcac141
1.2 atccta217
1.7 atccaa202
5.2 accctg246
7.6 aagcaa134
11.9 aagctc178+
18.4 atccaa112+
19.4 acacta131
22.7 atccca136
25.9 NsubA2F04+
27.1 atccaa245
28.0 NsubB278
36.1 aagcac132+
37.7 atcctg127
38.7 aagcac137+
39.4 NsubA262+
40.2 acacca169
43.0 atccac71
48.8 acacta237+
Nsub14
0.0 NsubC023+
7.2 acactc170
7.8 acccaa202
10.2 atcctg235
13.3 aagcat146
14.4 atcctg200
15.0 acacat312
15.3 atccaa180
16.6 NsubA249+acacac104
18.9 acccat216
19.9 atccta142
33.9 acactg61
37.7 aagcaa222
43.2 atccaa234
47.1 atccta120

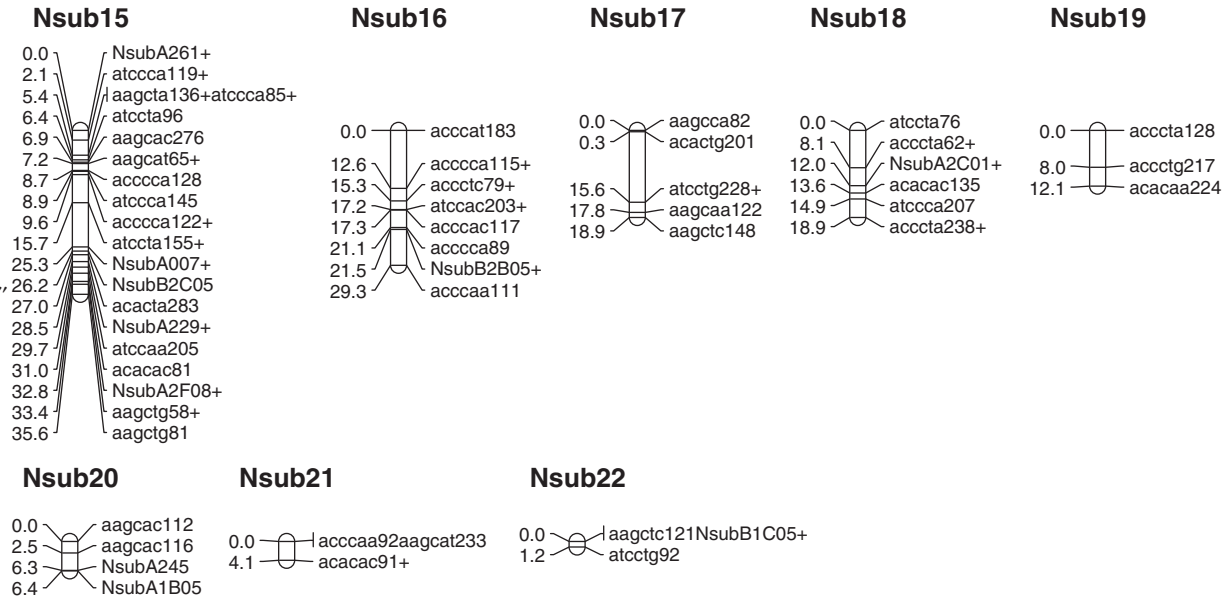


Fig. 1. Combined linkage map of *N. subnodosus*. Microsatellite markers are named with the prefix Nsub. Loci segregating in both parents (3:1 in the AFLP) are designated with a +. Haldane's map distances (cM) are noted.

scallop were fertilized with sperm from the latter. After spawning, a sample of adductor muscle was taken from each spawner and preserved in 75% ethanol.

Larvae culture was performed in 80-l tanks, feeding 30,000 cells/ml, increasing to 60,000 cells/ml, in water with salinity of 35 psu and at a temperature of 24 °C. Spat (juvenile scallops) were transported to the grow-out area in Estero de Rancho Bueno, Mexico, at three months of age. In November 2007, 1200 juveniles (>1 cm) from what would become the mapping family were placed into Nestier trays at the same location. Surviving individuals were recovered in February 2008 (~4 cm in length) and moved into culture bags for continued grow-out.

2.2. Phenotyping

At 14 months of age, all remaining individuals from the mapping family ($n=85$) were transported to the Aquaculture Genetics and Breeding Laboratory in La Paz, Mexico, for sampling. Length, height (hinge to distal tip), width (largest distance between left and right valves), total mass, soft tissue mass (mass without the shell), and adductor muscle mass phenotypes were recorded. Maturation stage was determined using the method of Sastry (1963), and the presence/absence of orange shell color was noted. After phenotyping, approximately 1 cm^3 of adductor muscle was placed into 467 µl of 1×TransPrep nucleic acid purification lysis buffer (Applied Biosystems Inc.) and 233 µl phosphate-buffered saline (PBS).

2.3. DNA extraction

Extraction of genomic DNA was performed at the Lucy Whittier Molecular and Diagnostic Core Facility (University of California Davis) using a 6100 semi-automated nucleic acid (ANA) workstation (Applied Biosystems Inc.) according to the manufacturer's instructions. Before extraction, the muscle tissue was homogenized using two, 4 mm, stainless steel beads in a GenoGrinder 2000 (SpexCertiprep). Protein was then digested from the homogenized lysate by incubating at 56 °C for 30 min with proteinase K and an additional 117 µl of PBS was added to 350 µl of the lysate before extraction took place.

Extracted DNA was quantified by staining 1 µl of extract with PicoGreen (Invitrogen), visualizing on a Fluorimager 595 (GE Healthcare), and comparing staining intensity to that of a dilution series of human genomic DNA of known concentration (Promega). Dilutions were performed when necessary to create a working stock of template DNA at 10 ng/µl.

2.4. Microsatellite genotyping

The parents of the mapping family were genotyped at all 73 available microsatellite loci (Ibarra et al., 2006; Petersen et al., 2009; and NsubA1B05, Genbank accession no. JN886991). With the exception of one locus that was amplified and scored individually, multiplex PCR reactions were developed incorporating from two to eight primer pairs per reaction. Each primer pair was identified by one of four fluorescent labels incorporated by 5′ end-labeling of the forward primer, or by using the tailing method of Schuelke (2000) (Supplementary Table 1). Multiple loci labeled with the same fluorescent dye were only multiplexed in cases where the parental alleles did not overlap in size. PCR conditions were as reported in Ibarra et al. (2006) and Petersen et al. (2009) with slight modifications to allow for multiplexing, which included 1 minute annealing and extension times, and additional cycles (33–38). Exact conditions for each multiplex are available from the authors upon request. PCR products from replicate samples (3–22 per marker) were scored in 49 loci to calculate the rate of genotyping error. PCR products were diluted with 70 µl of nanopure water. Of the diluted product, 1 µl was added to 8.8 µl of highly deionized (hi-di) formamide and 0.2 µl of LIZ600 size standard (both from Applied Biosystems Inc.) and denatured at 95 °C for 3 min. Electrophoresis of PCR products was performed on an ABI 3130xl Genetic Analyzer and allele scoring was performed in the software GeneMapper v4.0 (Applied Biosystems Inc.).

2.5. AFLP genotyping

AFLP of genomic DNA took place using a modification of Vos et al. (1995). Specifically, 100 ng of genomic DNA was digested with 1× T4 DNA ligase buffer, 5 U of restriction enzymes *Eco*RI and *Mse*I (New England Biolabs), and 1 Weiss unit of T4 DNA ligase (New England Biolabs). In the same digestion–ligation (dig–lig) step, 50 pmol of *Mse*I and 5 pmol of *Eco*RI adapters (prepared as in Agresti et al., 2000) were ligated in a reaction that also included 50 mM NaCl, 35 ng BSA,

1× T4 DNA ligase buffer, and nanopure water to a final volume of 16 μl. The dig–lig took place at 37 °C for 2 h and the products were consequently diluted with 84 μl of 1× TLE.

Pre-amplification was carried out using 1 μl each of 6 μM *Eco* + A and *Mse* + C primers, 0.45 U of Go Taq Flexi DNA Polymerase, 1× Go Taq Flexi buffer (both from Promega), 1.5 mM $MgCl_2$, 0.8 mM each dNTP (Promega) and 4 μl of the diluted dig–lig template in a total volume of 20 μl. Thermal cycler conditions included an initial denaturing step at 94 °C for 5 min followed by 25 cycles of 94 °C for 30 s, 56 °C for 1 min, and 72 °C for 1 min. The profile was completed by a final extension period of 10 min at 72 °C. The product was then diluted with 180 μl of 1× TLE.

Three base selective primers were used in the final, selective amplification. *Eco* + 3 primers were 5′ end-labeled with 6-FAM, PET, NED, or VIC. Selective amplification was performed using 3 μl of diluted pre-amp, 1× Multiplex Mastermix (Qiagen), two differently labeled *Eco* + 3 primers at a concentration of 3.85 μM each, one *Mse* + 3 reverse primer (unlabeled) at a concentration of 7.7 μM, and water to a total volume of 8 μl. Thermal cycler conditions for the selective amplification consisted of a 15 min initial denaturing at 94 °C, then 10 cycles of 94 °C for 30 s, 66 °C for 1 min, decreasing by 1 °C per cycle, and 72 °C for 1 min. Final cycling consisted of 18 cycles of 94 °C for 30 s, 56 °C for 1 min, and 72 °C for 1 min followed by a final extension step at 72 °C for 10 min. Resulting PCR product was diluted with 90 μl of nanopure water. Of the dilution, 1 μl of each sample was combined with 0.2 μl LIZ600 size standard and 8.8 μl highly deionized (hi-di) formamide (both from Applied Biosystems Inc.). The samples were denatured at 95 °C for 3 min before electrophoresis on an ABI 3730xl Genetic Analyzer. The 3730xl run module was modified to inject at 3 kV for 7 s to optimize the ability to distinguish and score peaks across the size range. AFLP fragments between 60 and 600 bp were scored in GeneMapper and named using the three selective bases of the *Eco*RI primer combined with the three selective bases for the *Mse*I selective primer, followed by the fragment size (bp). Loci segregating 1:1 (present and segregating in one parent only) were scored as well as those implied to be segregating in both parents (present in both parents and segregating ~3:1 in the progeny). Replicates of each step (four of digestion–ligation, two of pre-amplification, and two of selective amplification) were included throughout the process as well as one sample run in duplicate to check for consistency of electrophoresis. Any locus scoring inconsistently in any replicate was discarded.

2.6. Creation of a genetic map

AFLP and microsatellite genotypes were input into JoinMap4 (Van Ooijen, 2006) using the CP (cross pollination) population type. Loci that deviated significantly from Mendelian expectations at $\alpha \leq 0.0001$ as calculated by a chi-square test in JoinMap4 were eliminated from the analysis. To create linkage groups for loci segregating in only one parent, the threshold independence LOD score was set to 3.0 with all other settings left at default. After initial calculations were performed, ungrouped markers with a Strongest Cross Link (SCL) LOD score of greater than 3.0 were assigned to the linkage group suggested in a sequential manner. Phasing of the loci was performed by the software.

Placing the loci onto female and male maps was completed using the regression mapping option (Stam, 1993) and Haldane's mapping function with the linkage groups created in the prior step. In cases where the map order could not be determined using the default values (recombination frequency <0.4; LOD threshold >1), the stringency of the calculations was relaxed until a map order was determined. In addition to female and male maps, the complete data set was considered simultaneously to create a combined linkage map using a similar procedure with the exception of an increased threshold independence LOD score (5.0). Resulting maps were visualized in MapChart (Vorrips, 2002).

2.7. QTL mapping of size traits

Normality of the growth phenotypes was evaluated graphically with normality probability plots and statistically with Shapiro–Wilk tests using the Proc Univariate Normal command in SAS9.2 (SAS Inst). The software MapQTL6 (Van Ooijen, 2009) was used to detect significant regions for all growth traits using Kruskal–Wallis (KW) non-parametric tests and interval mapping (IM) with the regression mapping algorithm (Haley and Knott, 1992; Martinez and Curnow, 1992). Additionally, a principal component analysis (PCA) was performed on the six growth traits using PROC PRINCOMP in SAS9.2. The principal component was evaluated as a phenotype in the same manner as the raw trait data. Finally, due to its known correlation with adductor muscle mass and maturation stage (Barber and Blake, 2006), QTL analysis for muscle mass was repeated using maturation stage as a covariate in the model.

2.8. Mapping shell color

Linkage of the binary trait, color (presence or absence of orange), to microsatellites was evaluated using the software SOLAR (Almasy and Blangero, 1998) with calculations as described in Duggirala et al. (1997). The trait was also input into JoinMap4 as a dominant marker assuming orange color followed a recessive mode of inheritance and mapped concurrently with the complete AFLP and microsatellite data set.

3. Results

3.1. Genotyping

Of the seventy-three microsatellite loci available, it was determined by flanking region similarities and Blast (NCBI) analyses that six of these were duplicate markers and therefore excluded from the analysis (Supplementary Table 2). Genotyping of the remaining markers found a total of fifty-six microsatellites segregated in at least one of the parents of the mapping family, 50 markers in the male and 53 in the female; 47 of the markers were segregating in both parents. A total of 18 null alleles were identified in 15 loci by examining inheritance patterns in the progeny. Progeny genotypes were corrected to account for the inheritance of the null alleles where appropriate (null alleles were "scored" as 999 bp) and all loci remained in the analyses. Two scoring errors were found in 438 replicates for a microsatellite genotyping error rate of 0.46% and no evidence of self-fertilization was observed. Twenty-eight AFLP primer combinations resulted in 114 loci in the male and 116 in the female that segregated 1:1 (one parent heterozygous for the allele) and 86 segregating 3:1 (both parents heterozygous for the allele). The number of segregating loci per primer combination ranged from 3 to 17, averaging 11.3 (Supplementary Table 3).

3.2. Genetic map

Of the 165 segregating markers available for mapping in the female parent, 11 AFLP loci were removed due to segregation distortion and 3 AFLP loci removed due to similarity with other markers. In the female map 147 markers were placed into 27 linkage groups across a total map length of 919.71 cM; 22 linkage groups include microsatellites with six having only two markers (Table 1b and Supplementary Fig. 1). Stringency of mapping parameters was relaxed (recombination frequency <0.49 and LOD >0.7) for NsubF3, NsubF9, NsubF26, and NsubF27 to allow for marker order to be determined in those

Table 2
Comparison of recombination frequencies in the female (F) and male (M) germlines. The last column reflects the ratio of recombination rates between the germlines. The loci in the uppermost table show greater recombination in the female, while the opposite is true of those in the lower portion of the table.

Linkage group	Locus 1	Locus 2		Rec freq	F/M
Nsub1	Nsub2A1A06	NsubA1B12	Female	0.141	–
			Male	0.000	
Nsub1	NsubA1D10	NsubC261	Female	0.106	3.00
			Male	0.035	
Nsub3	NsubA2H05	NsubB1A02	Female	0.082	2.33
			Male	0.035	
Nsub4	NsubA274	NsubC262	Female	0.094	–
			Male	0.000	
Nsub4	NsubC020	NsubA274	Female	0.188	5.33
			Male	0.035	
Nsub13	NsubA2F04	NsubA262	Female	0.259	4.40
			Male	0.059	
Linkage group	**Locus 1**	**Locus 2**		**Rec freq**	**M/F**
Nsub2	NsubA1B09	NsubB235	Male	0.059	–
			Female	0.000	
Nsub3	NsubA001	NsubB1E11	Male	0.106	8.97
			Female	0.012	
Nsub3	NsubA1F07	NsubB1E11	Male	0.071	5.98
			Female	0.012	
Nsub3	NsubA1H09	NsubB1E11	Male	0.071	5.98
			Female	0.012	
Nsub9	NsubA1F03	NsubA1F04	Male	0.036	–
			Female	0.000	
Nsub15	NsubA229	NsubA2F08	Male	0.118	–
			Female	0.000	

linkage groups. The male parent had a total of 164 segregating markers available for map construction. Of these, one microsatellite and 13 AFLP loci were removed due to segregation distortion. The final map was 963.66 cM including 149 markers placed in 20 linkage groups, 19 of which were anchored by microsatellite loci (Table 1c and Supplementary Fig. 2). Mapping stringency was reduced in NsubM1, NsubM4, and NsubM15 to obtain a map order for these linkage groups.

In the combined data set, including the AFLP loci segregating 3:1, 22 linkage groups were identified after removing 29 AFLP loci that deviated significantly from Mendelian expectations and 3 AFLP loci that segregated identically to others in the dataset. The recombination and LOD stringencies were reduced in linkage groups Nsub1, Nsub2, Nsub8, and Nsub22 to obtain a map order of loci. In total, 320 loci were placed into linkage groups in the combined map covering 1124.14 cM (Table 1a and Fig. 1). Linkage groups for all maps were named in order of descending (estimated) length.

Twelve pairs of microsatellite loci were available for a comparison of recombination rates in the parents. No trend was observed when comparing rates of recombination between the female and male germlines; six pairs had higher recombination in the female parent while the opposite held true for the other six loci (Table 2). Considering only these pairs of loci, the average recombination rate in the female germline was 0.08, similar to the rate of 0.05 in the male.

3.3. QTL results

All growth traits were highly correlated, with Pearson Correlation Coefficients ranging from 0.83 between height and width to 0.96 between soft tissue mass and muscle mass (Table 3). The mean, maximum, minimum, and standard deviations of the phenotypes are also given in Table 3. Visual evaluation of normal probability plots (data not shown) suggested all traits adhered to the assumption of normally distributed residuals. However as determined by the Shapiro–Wilk test, residuals of the phenotypes length and width departed significantly from normality ($P = 0.02$ and 0.03, respectively) in this family. No change in results was observed after repeating the analysis with log transformed data for the length and width phenotypes (data not shown). Principal component analysis found the first principal component (PC) explained 91.7% of the variation in size phenotypes.

At the recommended threshold level of $\alpha = 0.005$ (Van Ooijen, 2009), only AFLP locus acactg91 remained significant in both the male and combined maps for the trait of shell width ($P = 0.005$ and 0.001, respectively), and microsatellite locus, NsubA2F04, in the female map for soft tissue mass ($P = 0.005$) (Table 4). Non-parametric analysis of the principal component also found linkage at $\alpha = 0.01$, but no markers reached the threshold of $\alpha = 0.005$ (Table 4).

Using IM, no loci for any phenotype met the threshold determined by permutation to indicate genome-wide significance. The QTL explaining the highest proportion of phenotypic variance (11%) in the male map was for shell width on linkage group NsubM10. This is supported by KW tests showing a significant marker (acactg91, KW $P = 0.005$) in that region. A LOD score reaching chromosome-wide significance for the trait of muscle mass was also found on male linkage group NsubM10 explaining 9.4% of the variance; when maturation stage was considered as a covariate, the LOD score for muscle mass increased from 1.81 to 1.89. However, with the exception of AFLP locus acactg91 with a p-value of 0.005, no marker within NsubM10 was significant using parametric tests at $\alpha = 0.01$ (Table 4). Finally, when considering the PC, interval mapping found a LOD score of 1.5 (chromosome-wide significance threshold 1.4) explaining 7.8% of the variance also on NsubM10; the most significant marker under KW tests on NsubM10 had a value of $P = 0.01$.

In the female map, IM found that one or both of linkage groups NsubF7 and NsubF9 had QTL for every growth trait significant at the chromosome-wide level (Table 4). However, only one LOD peak corresponded with markers found to be significant at $\alpha = 0.005$ using KW tests; this represents a putative QTL for soft tissue mass on female linkage group NsubF9 (LOD 1.57, chromosome-wide threshold = 1.1) explaining 8.1% of the phenotypic variance. Also found in similar regions on linkage group NsubF9 were significant LOD peaks for height, width, total mass, and muscle mass explaining 7.7–9.0% of the variance. The use of maturation stage as a model covariate for muscle mass decreased the LOD score, however, the most

Table 3
Pearson correlation coefficients between size phenotypes as well as the average, range, and standard deviation of each size phenotype in the mapping family.

	Correlation					Phenotype statistics			
	Length	Height	Width	Total mass	Soft tissue	Min	Max	Avg	Stdev
Length (cm)	–					4.00	6.60	5.65	0.47
Height (cm)	0.94	–				4.43	7.00	5.89	0.46
Width (cm)	0.87	0.83	–			1.53	3.10	2.52	0.26
Total mass (g)	0.94	0.93	0.89	–		17.40	76.80	48.60	11.19
Soft tissue (g)	0.93	0.90	0.86	0.95	–	5.00	23.30	14.16	3.50
Muscle (g)	0.89	0.85	0.85	0.90	0.96	1.10	10.70	5.22	1.64

Table 4
Results of QTL analyses for growth traits in the combined (Nsub), female (NsubF), and male (NsubM) maps. Shown are linkage groups with significant LOD scores in interval mapping and the associated KW parametric test scores of markers in the region at $\alpha \leq 0.05$.

Trait	Linkage group	Locus	KW	LOD peak[a]	Significance threshold[b]	Variance explained (%)
Length	NsubF7	aagcaa222	0.01	1.45	1.3	7.6
	NsubF9	NsubA2F04	0.05	1.54	1.2	8.0
Height	NsubF9	NsubA2F04	0.05	1.48	1.2	7.7
Width	NsubF9	NsubA2F04	0.01	1.74	1.2	9.0
	NsubM10	acactg91	0.005	2.15	1.4	11.0
	NsubM10	acacac73	0.05			
	NsubM10	Nsub2A1D12	0.05			
	NsubM10	aagctc243	0.05			
Total mass	NsubF7	aagcaa222	0.01	1.42	1.3	7.4
	NsubF9	NsubA2F04	0.01	1.65	1.1	8.5
Soft tissue	NsubF9	NsubA2F04	0.005	1.57	1.1	8.1
Muscle	NsubF9	NsubA2F04	0.01	1.56	1.2	8.1
	NsubM10	acactg91	0.01	1.81	1.3	9.4
	NsubM10	NsubA222	0.05			
	NsubM10	aagctc243	0.05			
Muscle — maturation as covariate	Nsub2		0.05[c]	3.63	3.2	16.5
	NsubF9		0.01[c]	1.42	1.2	6.8
	NsubM10		0.01	1.89	1.3	9.0
PCA	NsubF9	NsubA2F04	0.01	1.74	1.0	9.0
	NsubM10	acactg91	0.01	1.50	1.4	7.8
	NsubM10	NsubA222	0.05			

[a] The LOD score for the linkage group does not necessarily peak at the marker noted.
[b] Chromosome-wide.
[c] P-value of most significant marker in linkage group.

significant marker remained on NsubF9 where KW p-values ranged between 0.01 and 0.05 for these traits. Linkage group NsubF7 also showed chromosome-wide significant LOD peaks with corresponding KW significance at $\alpha = 0.05$ for length and total mass. The principal component mapped to NsubF9 with a LOD score of 1.74 (chromosome-wide threshold 1.0) and explained 9% of the variance while no single marker for the principal component was significant at $\alpha = 0.005$ (Table 4).

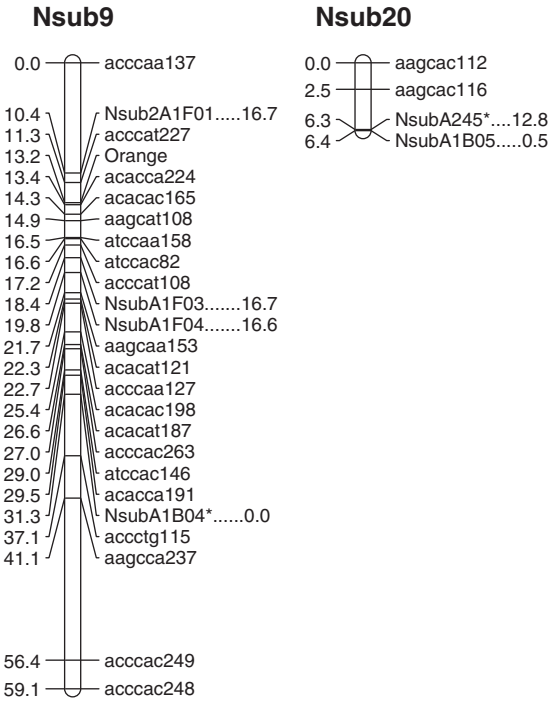


Fig. 2. Placement of orange on the combined linkage group, Nsub9, as determined by JoinMap4 assuming a recessive mode of inheritance. LOD scores are shown for the microsatellites as determined in SOLAR on both Nsub9 and Nsub20. * Indicates a two allele locus.

3.4. Shell color

Of the 85 progeny sampled in the mapping family, the shells of 26 (30.6%) were orange in color. There was no significant difference in growth phenotypes when comparing scallops with orange shell color to those without (data not shown). Evaluating the inheritance of shell color, SOLAR identified four highly significant microsatellite loci associated with orange pigment: NsubA1F04, Nsub2A1F01B, and NsubA1F03 on Nsub9, and NsubA245 on Nsub20. When treated as a dominant marker in JoinMap4, and assuming a recessive mode of inheritance, the phenotype mapped to Nsub9, 1.895 cM from AFLP locus acccat227 and 0.24 cM from acacca224 (Fig. 2).

4. Discussion

4.1. Genetic map

Thus far, linkage mapping of scallops has been limited to the bay scallop (Qin et al., 2007a,b), Zhikong scallop (Li et al., 2005; Wang et al., 2005), and an interspecific cross of *Chlamys* spp. (Wang et al., 2004). In all three cases, the maps relied heavily upon AFLP markers. Because the availability of codominant markers is limited, the success of AFLP was critical to creating the linkage groups in this study. In the prior studies, the lack of codominant markers such as microsatellites did not allow for integration of the male and female maps. Fortunately, the parents of this family were largely heterozygous; the large number of microsatellites segregating in the parents was critical to creating a combined map with a high level of genomic coverage.

The combined map of 22 linkage groups is three more than the haploid chromosome number ($n = 19$) (Ibarra et al., 2011). The female map has 27 linkage groups with six consisting of only two markers, and six not anchored by microsatellites (two of those, NsubF24 and NsubF17 also in the former category). The inclusion of the loci segregating in the male allowed some of these female linkage groups to be joined as seen in the combined map. Likewise, the female data help to combine two linkage groups in the male map. Additional markers and the genotyping of other mapping families in the future will help consolidate fragmented linkage groups and improve genome coverage.

4.2. Recombination

As functional hermaphrodites with few prior genetic studies, little is known about how recombination rate differs between the male and female germlines among individuals, and even between the male and female germlines within individuals. Overall, the male and female map lengths in this family were very similar to one another. This is contrary to a study of bay scallop by Qin et al. (2007a) who showed a male map distance 1.8× that of the female, though using a relatively inbred female line with fewer segregating markers than in the male. In oysters (Hubert and Hedgecock, 2004; Li and Guo, 2004; Yu and Guo, 2003) and abalone (Sekino and Hara, 2007), higher recombination rates have been documented in the female. However, Zhan et al. (2009) found rates varied among individuals more than between sexes in the Zhikong scallop. In the present mapping family, only twelve pairs of loci allowed for a comparison of recombination rates between the male and female meioses in the two spawners used. There was no consistent pattern across these loci. Sekino and Hara (2007) found a similar case in abalone, although they did note trends in certain chromosomes that favored higher recombination in the female and showed a great deal of variation in recombination rates between individuals of the same gender. Though more markers and families are needed to explore this phenomenon in the lion-paw scallop, the ability to create reciprocal crosses will allow for interesting future comparisons between the male and female germlines within an individual.

4.3. Mapping growth traits

Despite the failure of the traits shell length and width to adhere to the assumption of normally distributed residuals, log transformation of the data did not change the significance of the results. In addition, IM, especially using the regression algorithm, is robust to departures from normality (Van Ooijen, 2009) and nonparametric methods such as the KW test, do not assume a normal distribution. In this data set, interval mapping failed to identify any regions reaching genome-wide significance. As the spawners were wild-caught and not derived from inbred lines, the lack of highly significant LOD scores may reflect the heterogeneity of the genetic backgrounds of the parents. However, several instances were observed where LOD scores passed the threshold for chromosome-wide significance. While QTL for size in this study are preliminary, loci showing significance through KW testing and/or interval mapping should be considered in follow up studies of regions that may be contributing to growth phenotypes.

Using non-parametric testing in MapQTL6 (Van Ooijen, 2009), only three markers were significant at $\alpha = 0.005$ in the mapping family. However, several regions outside of these markers were identified by chromosome-wide significant LOD scores to be related to one or more growth traits. Of the markers shown to be significant via KW, the most robust is shell width on male linkage group NsubM10, also supported by a LOD score reaching chromosome-wide significance and accounting for 11% of the variance. This marker remains significant at $P = 0.001$ in the combined map. However, the LOD score with chromosome-wide significance on female linkage group NsubF9 for width does not correspond to the same markers and/or linkage group as in the male. While it appears the marker on the male map (acactg91) certainly contributes to the trait of shell width, the lack of significance in the same region of the female map and via interval mapping in the combined map creates uncertainty in whether the result represents a false positive, if there are additional loci involved in the phenotype, and/or if potential maternal or paternal effects are involved in determining the phenotype.

A similar situation is seen when examining the trait of muscle mass. A peak with chromosome-wide significance is observed in all three maps, but the significance does not correspond to the same markers across maps. The analysis of QTL for muscle mass was repeated with maturation state included as a covariate in the model because maturation is thought to correspond to changes in muscle composition (Barber and Blake, 2006). With this covariate included, LOD scores in the male increase slightly for NsubM10 (1.81 to 1.89) but decrease in the female LG NusbF9 (1.56 to 1.42). In the combined map however, Nsub2 becomes significant (LOD 3.63, chromosome-wide threshold 3.2), explaining 16.5% of the variance. This linkage group does not correspond to either linkage group suggested to be significant for this trait when considering the male or female maps individually, nor do any of the loci in Nsub2 have KW significance.

4.4. Mapping of the principal component

The principal component analysis was performed because of the high correlation between size traits. This strategy was also implemented by Qin et al. (2007a) in the bay scallop, which was successful in finding a QTL for growth explaining 11.5% of the variation. In this study, the first principal component, accounting for 91.7% of the variance, identified regions on the male and female maps associated with size. Not surprisingly, these were regions that were identified by interval mapping as significant in several of the individual phenotypes on female linkage group NsubF9 and male linkage group NsubM10. Taken all together, these data support the conclusion that NsubF9 and NsubM10 contain loci significant to the size of the scallop. In the combined map, a region of significance for the principal component was not detected and only one marker, acactg91, the same locus significant on NsubM10 in the male map, had a significance value of $P = 0.01$ using the KW test. Future studies should focus upon confirming the importance of these genomic regions for size traits.

4.5. Orange shell color

Shell color of the Pacific lion-paw scallop ranges from “neutral” off-white and various shades of brown, to purple, red, and orange. The “neutral” and orange shells are most common, with orange being found primarily in the Gulf of California populations (AMI, unpublished data). In the mapping family, 26 of the 85 scallops sampled (30.6%) were orange while all others were varying shades of brown. As the parents of the family were not noted as being orange, this suggests approximately a 3:1 ratio of non-orange to orange progeny, following an expected Mendelian inheritance pattern of a simple recessive trait. Similarly, a simple recessive mechanism appears to be responsible for the determination of shell color in the bay scallop, but with orange coloring dominant to white (Adamkewicz and Castagna, 1988; Qin et al., 2007b). A simple dominant locus is also believed to control light and dark color of oyster shells (Evans et al., 2009). However, not all shell colors studied are determined by a simple Mendelian mechanism. The Chilean scallop was found to have at least two loci involved in a dominant, epistatic interaction governing the shell color phenotype. Also in that study, the researchers were not able to determine the mechanisms of inheritance of the orange color, which did not appear to be under the control of a single gene (Winkler et al., 2001).

Examining only microsatellites, linkage group Nsub9 was identified to contain loci highly associated with orange shell color (LOD scores > 16). Further mapping of the phenotype as a dominant marker in JoinMap4 assuming a recessive mode of inheritance supported these results, positioning the trait on linkage group Nsub9 between AFLP loci acccat227 and acacca244. Interestingly, SOLAR also found a significant locus (NsubA245) on linkage group Nsub20 (LOD = 12.8). This is believed to be a false positive for two reasons. First, this locus contains only two alleles, and is not segregating in the male parent. With the assumed simple recessive mode of inheritance for the trait, segregation patterns at this locus would not allow for the detection of linkage to the trait. This is a similar case to locus NsubA1B04, found on the linkage group implicated as important in the genetic control of shell color, Nsub9. NsubA1B04 also has only two alleles and is not

significantly linked to orange (LOD = 0.0) although it lies within 11 cM of highly significant marker NsubA1F04. Secondly, locus NsubA1B05, a three allele marker that is tightly linked to NsubA245 on linkage group Nsub20, has a nonsignificant LOD score (LOD = 0.05). If NsubA245 (LOD = 12.8) were truly associated with the phenotype, this tightly linked marker should show significance as well. With the highly significant microsatellite loci on Nsub9 supported by independent placement of the phenotype using regression mapping methods, it seems certain that the marker for this trait is indeed on this linkage group. Nevertheless, to confirm both the position and mode of inheritance, more families will need to be investigated.

While the ultimate goal of QTL mapping in this scallop is to find major region(s) linked to growth, the identification of the linkage group linked to shell color may turn out to be a valuable resource for management. Due to the open water culture system of this species, it is a concern that hatchery propagated individuals could introgress with wild populations either through escape or the release of gametes. The determination of a marker controlling shell color could perhaps be used to create culture populations of all-orange scallops, which would allow for a new means of monitoring introgression and escape into the wild, both visually and genetically. This may especially be of interest in the Lagoon Guerrero Negro as orange scallops are infrequent in the waters of the Pacific. Future studies should focus on controlled crosses as well as the development of additional markers in this region to learn more about the genetic control of shell color.

5. Conclusion

In summary, this study shows that there is great potential for mapping QTL in the Pacific lion-paw scallop. With this F_1 family, linkage maps were created that have significant coverage of the genome and allowed for the identification of putative QTL for size characters. Selectively spawning additional families will help to confirm these or other regions that can be used in marker assisted selection. Finally, as functional hermaphrodites, the ability to perform reciprocal crosses will also allow for a unique opportunity to directly compare maternal and paternal controls of QTL, and differences in recombination rates.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.aquaculture.2012.03.039.

References

Adamkewicz, L., Castagna, M., 1988. Genetics of shell color and pattern in the bay scallop *Argopecten irradians*. The Journal of Heredity 79, 14–17.
Agresti, J.J., Seki, S., Cnaani, A., Poompuang, S., Hallerman, E.M., Umiel, N., Hulata, G., Gall, G.A.E., May, B., 2000. Breeding new strains of tilapia: development of an artificial center of origin and linkage map based on AFLP and microsatellite loci. Aquaculture 185, 43–56.
Almasy, L., Blangero, J., 1998. Multipoint quantitative-trait linkage analysis in general pedigrees. American Journal of Human Genetics 62, 1198–1211.
Baranski, M., Loughnan, S., Austin, C.M., Robinson, N., 2006. A microsatellite linkage map of the blacklip abalone, *Haliotis rubra*. Animal Genetics 37, 563–570.
Barber, B.J., Blake, N.J., 2006. Reproductive Physiology, Scallops: Biology, Ecology, and Aquaculture. Elsevier, Amsterdam, pp. 357–416.
Crenshaw Jr., J.W., Heffernan, P.B., Walker, R.L., 1991. Heritability of growth rate in the southern bay scallop, *Argopecten irradians concentricus* (Say, 1822). Journal of Shellfish Research 10, 55–63.
Deng, Y., Fu, S., Du, X., Wang, Q., 2009. Realized heritability and genetic gain estimates of larval shell length in the Chinese pearl oyster *Pinctada martensii* at three different salinities. North American Journal of Aquaculture 71, 302–306.
Duggirala, R., Williams, J.T., Williams-Blangero, S., Blangero, J., 1997. A variance component approach to dichotomous trait linkage analysis using a threshold model. Genetic Epidemiology 14, 987–992.
Evans, S., Camara, M.D., Langdon, C.J., 2009. Heritability of shell pigmentation in the Pacific oyster, *Crassostrea gigas*. Aquaculture 286, 211–216.
Haley, C.S., Knott, S.A., 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69, 315–324.
He, M., Guan, Y., Yuan, T., Zhang, H., 2008. Realized heritability and response to selection for shell height in the pearl oyster *Pinctada fucata* (Gould). Aquaculture Research 39, 801–805.
Hubert, S., Hedgecock, D., 2004. Linkage maps of microsatellite DNA markers for the Pacific oyster, *Crassostrea gigas*. Genetics 168, 351–362.
Ibarra, A.M., Ramirez, J.L., Ruiz, C.A., Cruz, P., Avila, S., 1999. Realized heritabilities and genetic correlation after dual selection for total weight and shell width in catarina scallop *(Argopecten ventricosus)*. Aquaculture 175, 227–241.
Ibarra, A.M., Petersen, J.L., Famula, T.R., May, B., 2006. Characterization of 35 microsatellite loci in the Pacific lion-paw scallop *(Nodipecten subnodosus)* and their cross-species amplification in four other scallops of the Pectinidae family. Molecular Ecology Notes 6, 153–156.
Ibarra, A.M., Alfonsi, C., Pérez, J.E., 2011. Genetics and biotechnology for aquaculture improvement and resource conservation of *Nodipecten* spp. In: Maeda-Martinez, A.N., Lodeiros-Seijo, C. (Eds.), Biologia y Cultivo de los Moluscos Pectinidos del Género Nodipecten. Editorial Limusa, S. A. de C.V. Grupo Noriega Editores, Balderas, pp. 135–147.
Lallias, D., Lapegue, S., Hecquet, C., Boudry, P., Beaumont, A.R., 2007. AFLP-based genetic linkage maps of the blue mussel *(Mytilus edulis)*. Animal Genetics 38, 340–349.
Langdon, C., Evans, F., Jacobson, D., Blouin, M., 2003. Yields of cultured Pacific oysters *Crassostrea gigas* Thunberg improved after one generation of selection. Aquaculture 220, 227–244.
Li, L., Guo, X., 2004. AFLP-based genetic linkage maps of the Pacific oyster *Crassostrea gigas* Thunberg. Marine Biotechnology 6, 26–36.
Li, L., Xiang, J., Liu, X., Zhang, Y., Dong, B., Zhang, X., 2005. Construction of AFLP-based genetic linkage map for Zhikong scallop, *Chlamys farreri* Jones et Preston and mapping of sex-linked markers. Aquaculture 245, 63–73.
Liang, J., Zhang, G.G., Zheng, H.P., 2010. Divergent selection and realized heritability for growth in the Japanese scallop, *Patinopecten yessoensis* Jay. Aquaculture Research 41, 1315–1321.
Maldonado-Amparo, R., Ramirez, J.L., Avila, S., Ibarra, A.M., 2004. Triploid lion-paw scallop *(Nodipecten subnodosus)*; growth, gametogenesis, and gametic cell frequencies when grown at a high food availability site. Aquaculture 235, 185–205.
Martinez, O., Curnow, R.N., 1992. Estimating the locations and the sizes of the effects of quantitative trait loci using flanking markers. Theoretical and Applied Genetics 85, 480–488.
Nell, J.A., Perkins, B., 2003. Evaluation of the progeny of third-generation Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) breeding lines for resistance to QX disease *Marteilia sydneyi* and winter mortality *Bonamia roughleyi*. Aquaculture Research 37, 693–700.
Perez, J.E., Alfonsi, C., 1999. Selection and realized heritability for growth in the scallop, *Euvola ziczac* (L.). Aquaculture Research 30, 211–214.
Petersen, J.L., Ibarra, A.M., Ramirez, J.L., May, B., 2008. An induced mass spawn of the hermaphroditic lion-paw scallop, *Nodipecten subnodosus*: Genetic assignment of maternal and paternal parentage. The Journal of Heredity 99, 337–348.
Petersen, J.L., Ibarra, A.M., May, B., 2009. Thirty-seven additional microsatellite loci in the Pacific lion-paw scallop *(Nodipecten subnodosus)* and cross-amplification in other Pectinids. Conservation Genetics Resources 1, 101–105.
Petersen, J.L., Ibarra, A.M., May, B., 2010. Nuclear and mtDNA lineage diversity in wild and cultured Pacific lion-paw scallop, *Nodipecten subnodosus* (Baja California Peninsula, Mexico). Marine Biology 157, 2751–2767.
Qin, Y., Liu, X., Zhang, H., Zhang, G., Guo, X., 2007a. Genetic mapping of size-related quantitative trait loci (QTL) in the bay scallop *(Argopecten irradians)* using AFLP and microsatellite markers. Aquaculture 272, 281–290.
Qin, Y., Liu, X., Zhang, H., Zhang, G., Guo, X., 2007b. Identification and mapping of amplified fragment length polymorphism markers linked to shell color in bay scallop, *Argopecten irradians irradians* (Lamarck, 1819). Marine Biotechnology 9, 66–73.
Ragone Calvo, L.M., Calvo, G.W., Burreson, E.M., 2003. Dual disease resistance in a selectively bred eastern oyster, *Crassostrea virginica*, strain tested in Chesapeake Bay. Aquaculture 220, 69–87.
Sastry, A.N., 1963. Reproduction of the bay scallop, *Aequipecten irradians* Lamarck. Influence of temperature on maturation and spawning. The Biological Bulletin 125, 146–153.
Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18, 233–234.
Sekino, M., Hara, M., 2007. Linkage maps for the Pacific abalone (Genus *Haliotis*) based on microsatellite DNA markers. Genetics 175, 945–958.
Shi, Y., Guo, X., Gu, Z., Wang, A., Wang, Y., 2010. Preliminary genetic linkage map of the abalone *Haliotis diversicolor* Reeve. Chinese Journal of Oceanology and Limnology 28, 549–557.
Stam, P., 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. The Plant Journal 3, 739–744.

Van Ooijen, J.W., 2006. JoinMap®4: Software for the Calculation of Genetic Linkage Maps in Experimental Populations. Kyazma BV, Wageningen, Netherlands.
Van Ooijen, J.W., 2009. MapQTL6: Software for the Mapping of Quantitative Trait Loci in Experimental Populations of Diploid Species. Kyazma B.V., Wageningen.
Vorrips, R.E., 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. The Journal of Heredity 93, 77–78.
Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23, 4407–4414.
Wang, S., Bao, Z., Pan, J., Zhang, L., Yao, B., Zhan, A., Bi, K., Zhang, Q., 2004. AFLP linkage map of an intraspecific cross in *Chlamys farreri*. Journal of Shellfish Research 23, 491–499.
Wang, L., Song, L., Chang, Y., Xu, W., Ni, D., Guo, X., 2005. A preliminary genetic map of Zhikong scallop (*Chlamys farreri* Jones et Preston 1904). Aquaculture Research 36, 643–653.
Winkler, F.M., Estevez, B.F., Jollan, L.B., Garrido, J.P., 2001. Inheritance of the general shell color in the scallop *Argopecten purpuratus* (Bivalvia: Pectinidae). The Journal of Heredity 92, 521–525.
Yu, Z., Guo, X., 2003. Genetic linkage map of the Eastern oyster *Crassostrea virginica* Gmelin. The Biological Bulletin 204, 327–338.
Yu, Z., Guo, X., 2006. Identification and mapping of disease-resistance QTLs in the eastern oyster, *Crassostrea virginica* Gmelin. Aquaculture 254, 160–170.
Zhan, A., Hu, J., Hu, X., Hui, M., Wang, M., Peng, W., Huang, X., Wang, S., Lu, W., Sun, C., Bao, Z., 2009. Construction of microsatellite-based linkage maps and identification of size-related quantitative trait loci for Zhikong scallop *(Chlamys farreri)*. Animal Genetics 40, 821–831.
Zhan, X., Fan, F., You, W., Yu, J., Ke, C., 2011. Construction of an integrated map of *Haliotis diversicolor* using microsat markers marine. Marine Biotechnology. doi:10.1007/s10126-011-9390-7.
Zheng, H., Zhang, G., Liu, X., Zhang, F., Guo, X., 2004. Different responses to selection in two stocks of the bay scallop, *Argopecten irradians irradians* Lamarck (1819). Journal of Experimental Marine Biology and Ecology 313, 213–223.