

A genetic linkage map of Pacific white shrimp (*Litopenaeus vannamei*): sex-linked microsatellite markers and high recombination rates

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Abstract Pacific white shrimp (*Litopenaeus vannamei*) is the leading species farmed in the Western Hemisphere and an economically important aquaculture species in China. In this project, a genetic linkage map was constructed using amplified fragment length polymorphism (AFLP) and microsatellite markers. One hundred and eight select AFLP primer combinations and 30 polymorphic microsatellite markers produced 2071 markers that were polymorphic in either of the parents and segregated in the progeny. Of these segregating markers, 319 were mapped to 45 linkage groups of the female framework map, covering a total of 4134.4 cM; and 267 markers were assigned to 45 linkage groups of the male map, covering a total of 3220.9 cM. High recombination rates were found in both parental maps. A sex-linked microsatellite marker was mapped on the female map with 6.6 cM to sex and a LOD of 17.8, two other microsatellite markers were also linked with both 8.6 cM to sex and LOD score of 14.3 and 16.4. The genetic maps presented here will serve as a basis for the construction of a high-resolution genetic map, quantitative trait loci (QTLs) detection, marker-assisted selection (MAS) and comparative genome mapping.

Keywords Linkage map · *Litopenaeus vannamei* · AFLP · Microsatellite · Sex

Introduction

Pacific white shrimp (*Litopenaeus vannamei*) is the leading species farmed in the Western Hemisphere (Meehan et al. 2003) and the most economically important shrimp species in China with the production of 300,000 metric tons in 2003, which accounts for 63% of all Chinese shrimp production (MAC (Ministry of Agriculture of China), China Agriculture Statistical Report 2003). Recently, sustainability of penaeid commercial fisheries and shrimp aquaculture industry has been threatened by overfishing, habitat destruction, and viral diseases such as those caused by Taura syndrome virus and White spot syndrome virus (Hasson et al. 1995; Pérez et al. 2005).

Research on reproduction, nutrition, immunity and pond management has supported shrimp industry growth, but genetic improvement programs leading to improved shrimp lines are only beginning to be applied. Much of the genetic research effort has been on the evaluation and selection of performance traits through selective breeding (Argue et al. 2002; Suárez et al. 2002; Donato et al. 2005). Heritabilities and genetic correlations for several important performance traits have been calculated (Perez and Ibarra 2003; Arcos et al. 2004; Gitterle et al. 2005a, b; Ibarra et al. 2005). To increase the efficiency of selection, particularly for complex traits such as disease resistance, feed conversion efficiency, growth rate and pond survival, trait-linked DNA markers are needed. Research on *L. vannamei* genomics is developing rapidly, more than

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9000 sequences were accumulated in public databases (as of October 2005; <http://www.ncbi.nlm.nih.gov>), several complete genes were cloned and their expression patterns were reported (Yang et al. 2003; Lai et al. 2005), physical map construction were undertaken in our lab.

A primary genetic linkage map is an essential prerequisite to detailed genetic studies in any organism and linkage maps have been developed for several aquaculture species (Kocher et al. 1998; Waldbieser et al. 2001; Woram et al. 2004; Moen et al. 2004; Hubert and Hedgecock 2004; Chistiakov et al. 2005; Kai et al. 2005; Lee et al. 2005). High-density linkage maps are necessary for efficient mapping of quantitative trait loci (QTLs) to complement marker-assisted selection (MAS) and for comparative genome mapping (Lander and Botstein, 1989). However, a fine linkage map requires large numbers of molecular markers. Amplified fragment length polymorphism (AFLP; Vos et al. 1995), being highly reliable and informative, can generate a lot of markers without any prior exploration of the genome, presents an ideal method to study organisms with limited genome information and resources such as penaeid shrimp. Microsatellites, a type of codominant markers, may transfer easily to new crosses and populations, even to different species for those EST derived markers, it is, however, time-consuming and expensive. In *L. vannamei*, more than 200 microsatellite markers have been developed (Meehan et al. 2003; Pérez et al. 2005), but none of them has been mapped.

In the present study, two sex-specific genetic linkage maps of *L. vannamei* were constructed by analyzing the segregation of AFLP and microsatellite markers on 94 progeny with automatic capillary genotyping, and sex-linked microsatellite markers were mapped on the maternal map.

Materials and methods

Resource families

Two hundred full-sib families were produced in our breeding base of Hainan in 2003, and all parents were either specific pathogen-free SPF *L. vannamei* produced in USA or their descendants. 04 cross was selected as the mapping family because it was relatively more informative, which means that 04 cross always produce relatively more polymorphic loci when the same AFLP primer combination or microsatellites were used. DNA was prepared from the parents and 94 progeny.

AFLP analysis

DNA was extracted using the Qiagen DNeasy Tissue kit following recommended protocols. AFLP analysis was carried out as described by Li and Guo (2004), which was originally derived from Vos et al. (1995). The digestion–ligation reaction mixture contained 3 µl genomic DNA (about 200 ng), 1.1 µl 10 × T4 DNA ligase buffer with ATP, 1.1 µl 0.5 M NaCl, 0.55 µl of 1 mg/ml bovine serum albumin (BSA), 1 U *MseI*, 5 U *EcoRI*, 1.0 U T4 DNA ligase, 50 pmol *MseI* adaptor, 5 pmol *EcoRI* adaptor and water to bring the final volume to 11 µl. The reaction mixture was incubated at 37°C for 2 h and then diluted with 150 µl TE_{0.1} (20 mM Tris–HCl, 0.1 mM EDTA, pH 8.0). Preselective amplification was performed using primers complementary to the adaptor sequence without any additional nucleotides at the 3' end. Selective primers consisted of the preselective primers with three selective nucleotides added to the 3' end. *EcoRI*-selective primers were fluorescently 5' labeled with FAM. PCR reactions were carried out in a PTC-100 or PTC-200 thermal cycler (MJ Research). PCR products labeled with FAM dye were analyzed with an ABI Prism 3100 Avant DNA analyzer (Applied Biosystems).

AFLP markers were named after the primer pairs used to generate them and their size. *EcoRI*- and *MseI*-selective primers were coded by letters and numbers, respectively (Table 1), followed by a letter f (fragment) and digits representing the size in base pairs (Li et al. 2005). For example, B8f160 refers to the 160-bp fragment generated by the *EcoRI* primer B (ACA), and the *MseI* primer 8 (CAT).

Microsatellite genotyping

Microsatellite were scored using 0.2 µM dye-labeled (5-FAM) forward primers and 0.5 µM reverse primers. The PCR reaction was performed in a PTC-100 or PTC-200 thermal cycler (MJ Research) using a first incubation of 94°C for 4 min, followed by 30 cycles of 94°C for 20 s, annealing time for 30 s and 72°C for 30 s. A final extension of 15 min at 72°C was added. Products were separated on POP4 gel with an ABI Prism 310 sequencer or an ABI 3100 Avant DNA analyzer (Applied Biosystems).

We tested 100 microsatellite loci recently described by Meehan et al. (2003) and Ball et al. (1998) for this study, finding 30 informative (Table 2). Because most of the original name is too long to be permitted when using the mapping software MapMaker/Exp v. 3.0, microsatellite markers were renamed according to information of the species, experimental order in our

Table 1 Matrix identification of AFLP primer combinations in *L. vannamei* and the mapped segregating markers (before slash), along with the total segregating markers (the slash)^a

	E-AAG(A)	E-ACA-(B)	E-ACG(D)	E-AGC(E)	E-AGG(F)	E-CCG(G)	E-CCT(H)	E-CTC(I)	E-TAC(J)	Total
M-ACA:1					8/27(11)	1/16(1)	2/24(17)	1/8(4)	12/75(33)	
M-ACC:2		6/17(2)		8/18(3)	5/14(3)			2/13(8)	21/62(16)	
M-ACT:3		5/23(9)	2/12(5)	4/18(7)					16/72(28)	
M-ATC:4	9/25(9)							4/25(9)	21/69(25)	
M-CAA:5	10/26(15)		5/13(3)	3/11(6)	11/27(11)	1/19(9)		11/29(12)	43/141(60)	
M-CAC:6	12/26(12)			3/15(8)	4/23(13)	4/23(13)		4/20(7)	23/84(40)	
M-CAG:7	13/25(12)	10/24(4)	3/8(1)	8/22(7)	6/31(13)	3/21(10)	2/17(9)		46/176(70)	
M-CAT:8	12/30(10)	3/13(9)	4/17(9)	8/25(8)	8/20(5)	7/16(4)	12/35(11)	5/19(12)	59/175(68)	
M-CCA:9	14/26(8)		6/16(6)		9/17(3)		7/16(4)	1/6(3)	42/98(31)	
M-CCG:10	2/17(9)	8/13(1)	4/11(4)	5/16(5)		0/13(8)	3/22(8)		22/92(35)	
M-CCT:11	8/30(14)						6/17(7)		17/57(27)	
M-CGA:12			4/12(5)		4/20(10)		2/17(12)		4/34(23)	
M-CGC:13		5/13(2)	2/10(4)		2/18(13)		1/14(6)		14/59(23)	
M-CGG:14		1/11(3)			4/20(8)				4/28(17)	
M-CGT:15				10/18(3)			7/24(7)		22/73(21)	
M-CTA:16	2/7(2)		5/25(11)	6/21(9)		0/10(7)		2/10(6)	20/84(40)	
M-CTC:17	4/17(6)		5/15(8)	8/24(10)	10/28(12)	10/16(4)		2/14(7)	52/148(56)	
M-CTG:18			8/13(1)			3/14(6)			25/68(20)	
M-CTT:19	8/21(4)				7/26(12)	4/20(8)	11/39(20)	11/48(16)	46/170(65)	
M-GAA:20								0/18(10)	0/18(10)	
M-GAC:21	2/19(9)								2/19(9)	
M-GCC:22	3/13(5)							7/26(10)	12/63(29)	
M-GTA:23								6/23(8)	10/55(26)	
M-TAA:24	0/8(4)								0/8(4)	
M-TAC:25	2/13(4)								2/13(4)	
M-TAG:26	6/24(16)							0/16(5)	6/40(21)	
M-TCA:27	2/19(8)							8/22(8)	10/41(16)	
M-TGA:28	1/11(5)								1/11(5)	
Total	110/357(152)	38/114(30)	48/152(57)	63/188(66)	74/248(101)	32/152(69)	44/178(73)	64/297(125)	552/2033(822)	

^a The skewed segregating markers were in the parenthesis. E-TAA was coded as K (Only one combination was used with this fluorescence primer, the corresponding numbers is 1/8(4).)

analysis, and the size of the microsatellite bands (Table 2). The first letter of the marker indicates the species (e.g., v for *L. vannamei*), followed by digits representing analyzing order in our work and the size of the microsatellite band similar with the AFLP markers. For instance, microsatellite marker v1f148 indicates that the microsatellite marker of *L. vannamei* was analyzed first in our experiments with a size of 148 bp.

Linkage map construction

Maps were constructed using a two-way pseudotest-cross design (Grattapaglia and Sederoff 1994). Segregating markers were tested for deviations from expected 1:1 or 1:1:1:1 segregation ratios by χ^2 analysis ($P < 0.05$). Marker data showing no significant deviations were used to calculate linkage. Their segregation type was coded as backcross with the banded genotype as heterozygous (H) and non-banded as homozygous recessive (A). Two separate data sets were obtained

for the maternal and paternal parents. Each data set was first entered in the mapping software MapMaker/Exp v. 3.0 (Lander et al. 1987) following the F2 backcross model, then duplicated by changing A to H and H to A. The reciprocal data sets detect linkage of markers by creating two identical linkage groups (Li et al. 2005).

Markers were sorted into the linkage groups with an initial threshold LOD score of 3.0 and maximum genetic distance of 35 cM. For linkage groups with nine or fewer markers, the most likely order within each group was determined using the COMPARE command. For all other groups, the THREE POINT and ORDER commands were employed to obtain the order of markers with unique placement, followed by the TRY command to find the most likely placement of the remaining markers, and subsequent orders were tested using the RIPPLE command. 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 option, and the map distances in centi-Morgan (cM) were calculated in Kosambi's mapping function (Kosambi 1944). Maps were drawn using MapChart software (Voorrips 2002). Linkage groups were assigned in descending size, and distorted markers were suffixed with an asterisk.

To study the marker distribution along the map, we used the χ^2 -test for goodness of fit as described by Rouppe van der voort et al. (1997) to test whether the mapped markers were randomly distributed within a linkage group. We also used the Kolmogorov–Smirnov and Lilliefors one-sample test (Kolmogoroff 1941; Lilliefors 1967) (on standardized data) to compare the distribution of marker intervals between consecutive loci along the map against the null expectation that they follow a normal distribution (Wang and Porter 2004). The marker distribution was also analyzed by calculating the Pearson correlation coefficient between the number of markers in the linkage groups and the size of the linkage groups (Sokal and Rohlf 1981). The marker distribution was tested on framework markers.

Estimation of map length and map coverage

Both map length and map coverage were calculated based on framework markers. Three approaches were used to estimate the map length of *L. vannamei*: (1) G_{e1} : The genome was estimated by simply adding 2s (s is average spacing of the linkage map) to the length of each group to compensate the two chromosome ends beyond the terminal of each linkage group

Table 2 Informative microsatellite markers in *L. vannamei*^a

Renamed microsatellite	Corresponding markers in The original conference	Annealing temperature (°C)	GenBank accession number
v1	TUMXLv3.1	57	AF360017
v2	TUMXLv8.220	57	AF360072
v5	TUMXLv5.27	57	AF360022
v6	TUMXLv6.23	60	AF360031
v9	TUMXLv9.43	60	AF360109
v26	TUMXLv5.45	54	AF360025
v29	TUMXLv7.56	62	AF360055
v31	TUMXLv7.121	56	AF360043
v33	TUMXLv7.148	54	AF360051
v43	TUMXLv8.256	55	AF360076
v90	TUMXLv9.103	55	AF360090
v92	TUMXLv10.33	55	AF359992
v95	TUMXLv10.220	59	AF359969
v98	TUMXLv10.312	59	AF359989
v110	Pse036	55	AF047361
v113	TUMXLv5.35	52	AF360023
v114	TUMXLv6.63	55	AF360040
v117	TUMXLv7.97	59	AF360057
v119	TUMXLv8.9	55	AF360089
v120	TUMXLv8.176	50	AF360063
v122	TUMXLv9.28	59	AF360108
v123	TUMXLv9.60	50	AF360111
v126	TUMXLv9.145	55	AF360098
v129	TUMXLv9.174	59	AF360104
v130	TUMXLv9.178	55	AF360105
v133	TUMXLv10.27	55	AF359979
v143	TUMXLv10.201	59	AF359959
v145	TUMXLv10.208	55	AF359964
v150	TUMXLv10.278	50	AF359980
v155	TUMXLv10.343	52	AF359996

^a Most of the microsatellite were from Meehan et al. (2003), however v110 was from Ball et al. (1998)

(Fishman et al. 2001); (2) G_{e2} : Each linkage group was multiplied by the factor $(m + 1)/(m - 1)$, and m is the marker number on each linkage group (i.e., disregarding markers mapping to the same place). The estimated map length is the sum of revised length of all linkage groups (Chakravarti et al. 1991); and (3) G_{e3} : The genome was estimated by the formula $N(N - 1)X/K$, where N is the number of framework makers, X is the maximum interval between two adjacent markers on the framework map at a certain minimum LOD score (3 in this study), and K is the number of marker pairs at the same LOD score, which is obtained by the command BIG LOD (Cervera et al. 2001). The final estimated map length (G_e) is the average of the estimated map length using these three approaches. The observed map length was calculated as the length of the framework map (G_{of}) and the total length (G_{oa}) considering all the markers on the framework map, the triplets and doublets. The map coverages, C_{of} and C_{oa} , were calculated as G_{of}/G_e and G_{oa}/G_e , respectively (Li et al. 2005).

Results and discussion

Linkage maps

A total of 2,071 polymorphic loci were produced with the 108 *EcoRI/MseI* primer combinations and 30 microsatellite markers. Among the AFLP polymorphic markers, 826 markers (40.5%) were not used for the construction of the linkage map because they showed a significant distortion from the expected 1:1 ratio at $P = 0.05$ level. All the microsatellite markers were used to calculate linkage, and three of them deviated from expected 1:1 or 1:1:1:1 segregation ratios ($P < 0.05$). The remaining 1,245 markers were analyzed using the Mapmaker program. A total of 816 markers were assigned to the two sex-specific linkage maps. Among the remaining AFLP markers, 267 were unlinked, and 162 were eliminated because they showed uncertain linkage or spanned very large map distances.

The female data set was composed of 642 segregating markers, and the male data were composed of 603 segregating markers. Among the 642 female markers, 319 (including 18 microsatellite loci) were mapped to the female framework map (Fig. 1) that contained 45 linkage groups that ranged in size from 28.5 cM to 260.0 cM and consisted of between 4 and 16 markers (Fig. 1, Table 3). The total linkage distance spanned by these markers was 4134.4 cM, and the spacing of each group ranged from 7.6 cM to 25.9 cM

with an average of 15.1 cM for the whole framework map. Most of the consecutive marker spacing was less than 30 cM. Twenty-three smaller linkage groups were identified that ranged in size from 3.1 cM to 69 cM and consisted of 12 triplets and 11 doublets. Four of these linkage groups contained one microsatellite marker and spanned a total of 99.7 cM; 19 additional linkage groups that consisted of only AFLP markers spanned a total of 541.2 cM. The total length of the map including the triplets and doublets, was 4775.3 cM. One hundred and sixty-eight markers (160 AFLP and eight microsatellite) did not show linkage to any other marker and were not assigned to any of the linkage groups. Seventy-six markers were eliminated because they showed uncertain linkage or spanned very large map distances.

Among the 603 male markers, 267 were assigned to 45 linkage groups, with 252 AFLP loci and 14 microsatellite loci placed at precise positions (Fig. 1) covering 3220.9 cM. The length of each group varied from 14.2 cM to 161.1 cM Kosambi. The spacing of each group ranged from 4.1 cM to 25.5 cM with an average of 14.5 cM for the whole framework map. Most of the consecutive marker spacing was also less than 30 cM. Forty linkage groups were identified that consisted of markers less than 4. Three of these linkage groups contained at least one microsatellite marker and spanned a total of 52.7 cM; 37 additional linkage groups that consisted of only AFLP markers spanned a total of 906.6 cM. The total length of the map including the triplets and doublets was 4180.2 cM. There were seven common microsatellite markers: v155, v117, v113, v133, v98, v1 and v123 between female and male map, they were located in linkage group 3, 5, 11, 12, 20, 29 and 33 in the female map, and linkage group 8, 29, 2, 6, 43, 34, 1 in the male map, respectively. The parameters of the two maps are summarized in Table 3.

Compared with the total mapped markers (only AFLPs, $LOD > 3$) of 155 and 135 in the preliminary *L. vannamei* map reported by Pérez et al. (2004), there are 319 markers for the female and 267 for the male in the maps presented here, including 18 and 14 microsatellite markers, respectively. Without the male parent, the maternal parental genotypic data were used as the references to deduce the information from the male progenitor, along with the small mapping population of 42 in the map of Pérez et al. (2004), markers segregation type may be confused, which means that some 1:1 markers may be regarded as 3:1, and vice versa.

The haploid genome of *L. vannamei* has 44 chromosomes (Ramos 1997), and therefore, the numbers of linkage groups are expected to be equal to the

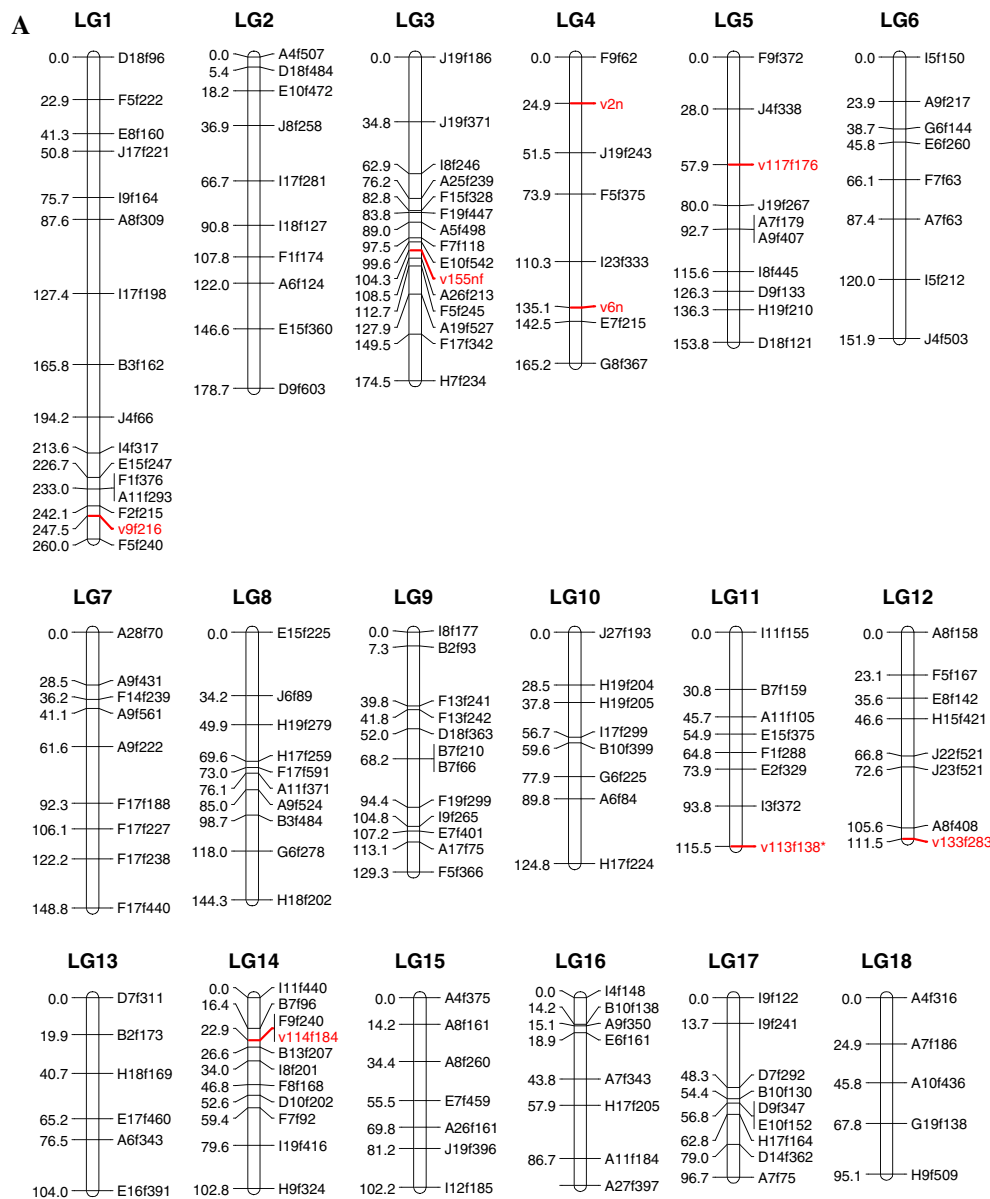


Fig. 1 The female (**A**) and male (**B**) linkage maps of the Pacific white shrimp. Markers are shown on the right of each group, and the adjacent marker spacing is displayed on the left in Kosambi

chromosome number. Our study produced 45 linkage groups for both the maternal and parental maps. It is obvious that some linkage groups belong to the same chromosomes. This observation, together with observations of small linkage groups, and several unlinked AFLP and microsatellite markers, suggests that gaps remain to be filled by adding more markers. Other enzyme combinations such as *PstI*–*MseI* may increase the density of the map and fill gaps. Obviously, AFLPs are dominant markers that provide less genetic information as compared to microsatellite markers. Mapping of additional codominant markers such as

cM. The distorted markers were marked by the suffix symbol “*”, and the microsatellite markers were marked by red color

microsatellite and SNPs to the AFLP-based map should eventually bring the number of linkage groups to the number of chromosomes.

Genome estimation, map coverage and map differences between sexes

Based on the framework markers, the map length was estimated as 5487.6, 5514.6 and 5331.7 cM with the three approaches with the average of 5444.6 cM for the female; In the male, the estimated map length was 4538.1, 4514.5 and 4826.1 cM with the average of

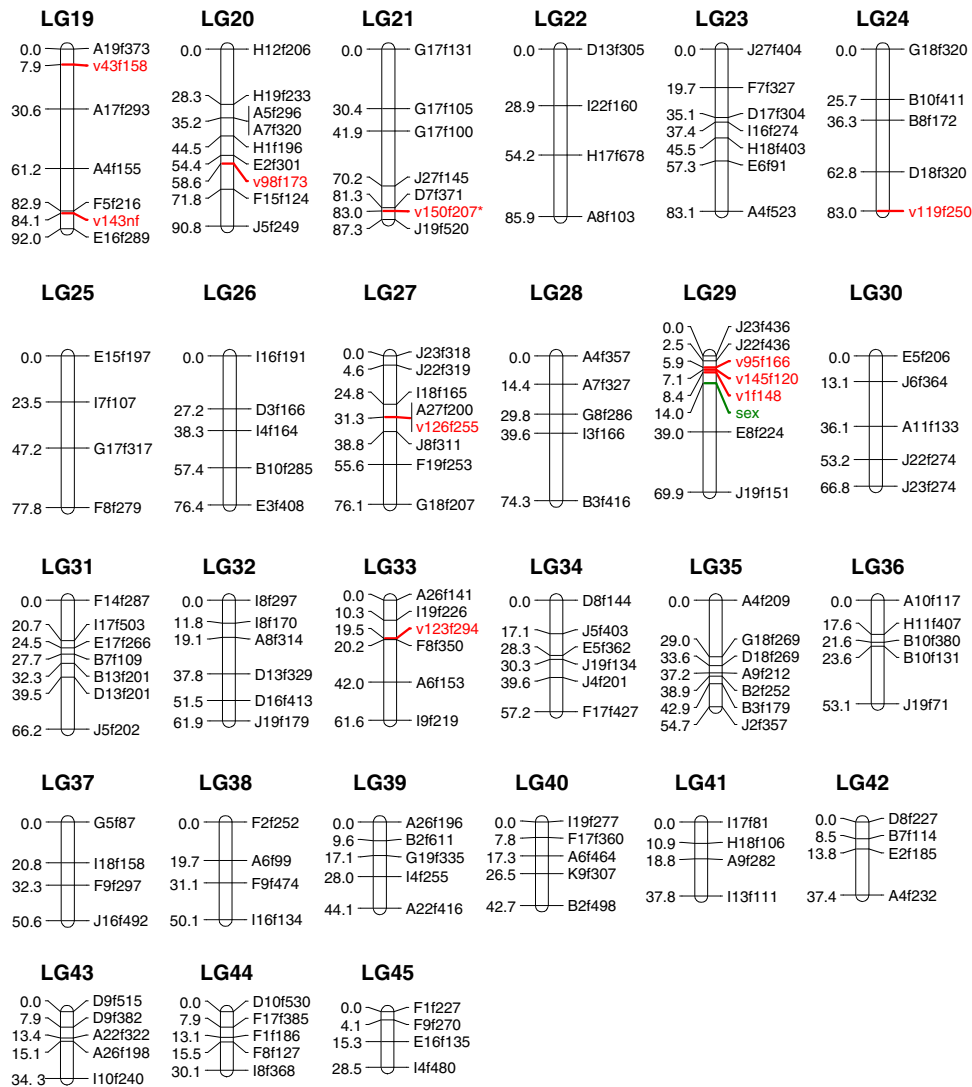


Fig. 1 continued

4626.2 cM. Based on the observed length of the framework map (4134.4 cM and 3220.9 cM) and the estimated map length (5444.6 cM and 4626.2 cM), the framework map had a coverage of 75.9% and 69.6% in the female and male, respectively. When considering all the triplets and doublets, the map coverage increased to 87.7% for the female and 90.4% for the male (Table 3).

The map length of *Marsupenaes japonicus* was 2300 cM (Moore et al. 1999), while that for *L. vannamei* was 2771 cM for the female and 2116 cM for the male maps, and the estimated map length was 4445 cM and 3583 cM with the more conservative estimates (Pérez et al. 2004). Nevertheless, the estimate in *M. japonicus* was made using a different technique (bootstrapping) that might give different results with low genome coverage. However, Li et al. (2003) report

a second-stage map with increased coverage in *M. japonicus*, with a total length of 1780 cM for the male map that is close to the that estimate given by Moore et al. (1999). In our linkage map of *L. vannamei*, the observed and estimated map lengths are much longer than reported by Pérez et al. (2004) because of our improved map coverage. Genotyping errors and missing data cause an upward bias in size estimates, and non-random distribution of markers with respect to recombinational distances causes downward bias (Chakravarti et al. 1991). MAPMAKER itself may expand the map (Sybenga 1996). The estimated size of the penaeid prawn genome is approximately 2×10^9 bp (Chow et al. 1990), which is two-thirds that of the human genome (about 3×10^9 bp and 3000 cM). In *L. vannamei*, extra linkage groups, many triplets, doublets and the estimated genome size indicate that

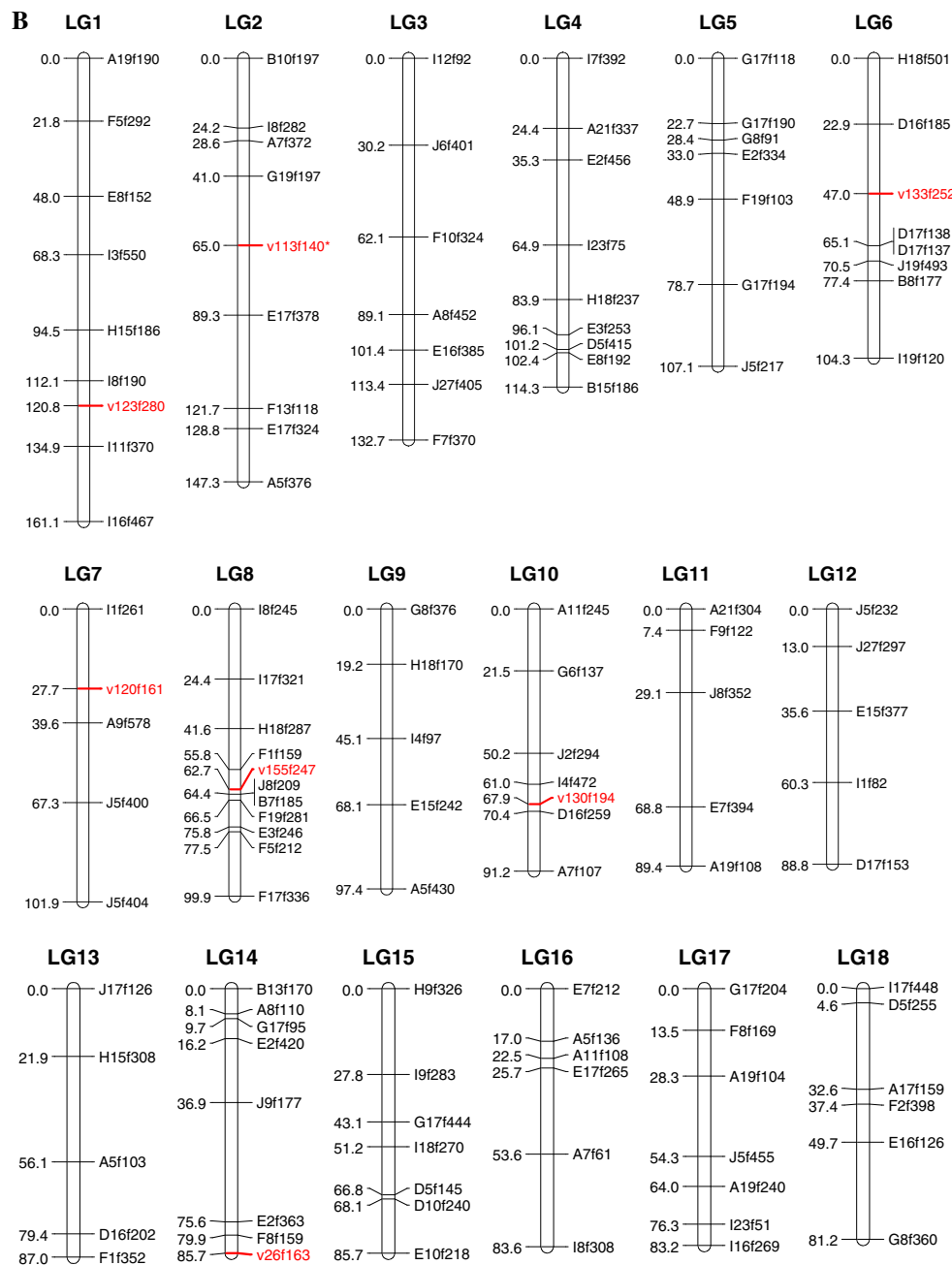


Fig. 1 continued

our maps need to be improved. Our total map length for both the male and female maps show evidence that the recombination rates in *L. vannamei* are higher than those in closely related species.

In human, mouse, cattle, pig, fish and, indeed, most animals studied so far, recombination rates show a significant difference between sexes. Female maps are usually longer than male maps (Sakamoto et al. 2000; Singer et al. 2002; Hubert & Hedgecock 2004; Moen et al. 2004; Woram et al. 2004). The molecular mechanism responsible for the differences in recombination

rates between the two sexes is not currently well understood. In *L. vannamei*, as in other species, the female map is longer than the male map, although we cannot localize differences in the recombination rates to centromeres or telomeres.

Haldane (1922) and Huxley (1928) noted that when meiotic recombination rates vary between the two sexes, it is usually the heterogametic sex that has suppressed recombination. In mammals, many species show reduced recombination frequency in males, which are the heterogametic sex. Although the

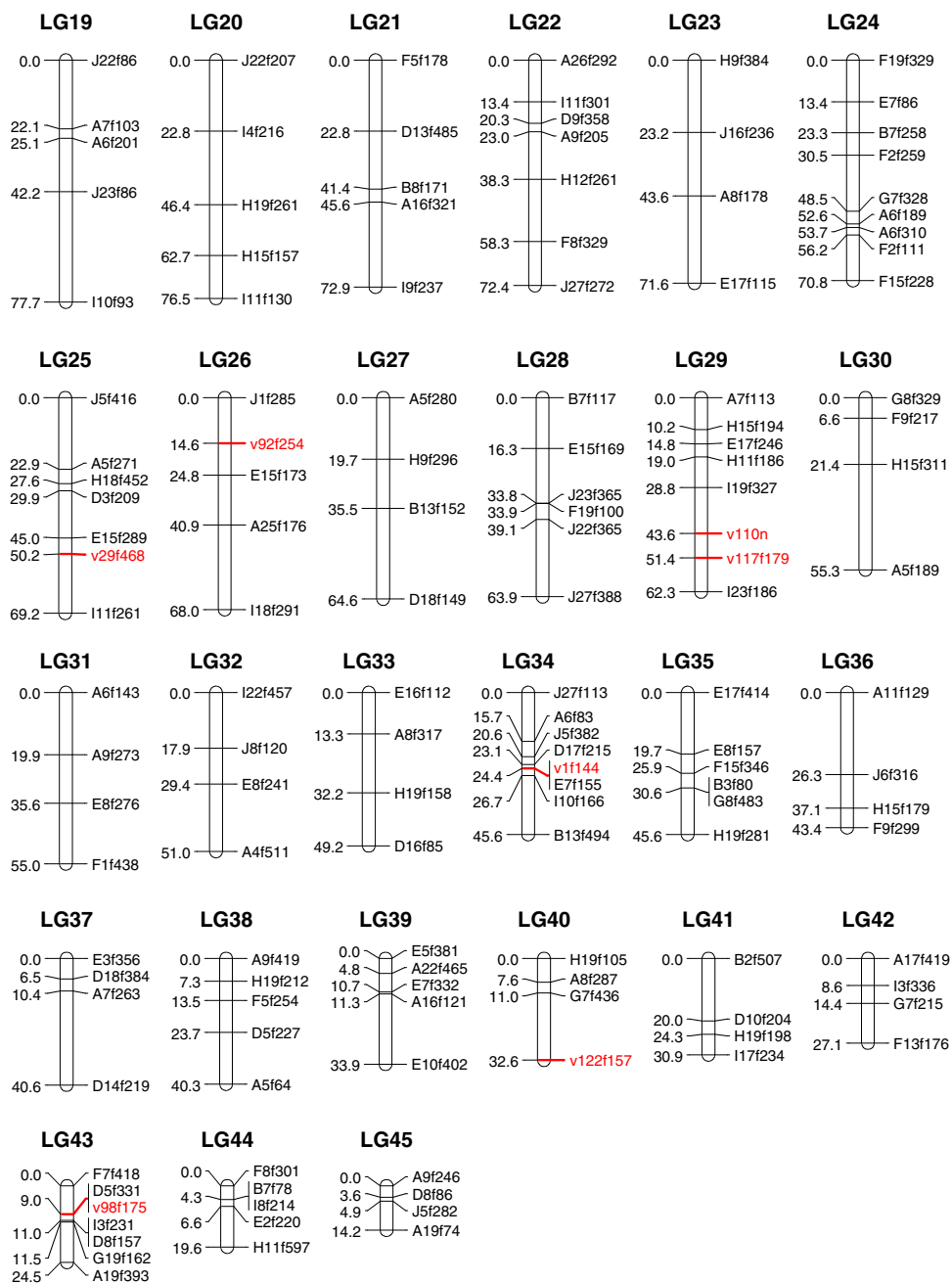


Fig. 1 continued

heterogametic sex usually has reduced recombination, there are exceptions to the rule. For example, in sheep, rams have 1.3 times as much recombination as ewes (Crawford et al. 1995), and in cattle there is no significant sex-specific difference (Kappes et al. 1997). All species of penaeid shrimp do not show heteromorphic sex chromosomes, so the rule does not apply. Recent selection history may also influence the rate of recombination within a strain, and increased levels of recombination have been reported in strains undergoing intense selection (Korol et al. 1994).

Marker distribution

Overall, AFLP markers were randomly distributed in the linkage maps, as indicated by the significant ($P < 0.01$) correlation between the number of markers in the linkage groups and the size (length) of the linkage groups for both female and male maps. Kolmogorov–Smirnov and Lilliefors one-sample test also indicated that intervals between consecutive markers showing a normal distribution in the female map ($P = 0.06$), while non-normal in the male data

($P = 0.03$). Some small clusters and gaps were noticeable in both maps. However, no clustering was revealed by the chi-square test for goodness of fit ($P < 0.05$) described by Rouppe van der Voort et al. (1997). The test, however, is effective only for groups with >10 markers, which is not the case for a majority of our linkage groups.

Markers may distribute randomly in some species (Castiglioni et al. 1999; Cervera et al. 2001), but form clusters in others (Young et al. 1998; Sakamoto et al. 2000; Liu et al. 2003). The distribution of mapped markers in the Pacific white shrimp is mostly random. The reasons for the high level of marker clustering are not known at present. Some potential causes include: a reduced recombination rate around centromere regions and/or telomere regions, hot spots of recombination, a *bona fide* enrichment of AFLP markers in these regions due to the uneven distribution of restriction sites, and the presence of highly repetitive elements (Liu et al. 2003). Markers tend to cluster around regions where recombination is suppressed, usually corresponding to centromere and telomere regions (Tanksley et al. 1992; Rouppe van der Voort et al. 1997). Because only *EcoRI* and *MseI* were used as restriction enzymes for AFLP analysis, there is a chance that the AFLP markers are directed toward AT-rich regions, leaving a void of markers in GC-rich regions.

Table 3 Summary of the female and male linkage maps of the *L. vannamei*

	Female	Male
Linkage groups	45	45
Average number of markers per group	7.1	5.9
Minimum marker number per group	4	4
Maximum marker no. per group	16	11
Average marker spacing (cM)	15.1	14.5
Maximum marker spacing (cM)	39.8	38.7
Minimum length of linkage group	28.5	14.2
Maximum length of linkage group	260.0	161.1
Unlinked triplets	12	19
Unlinked doublets	11	21
Sex	1	0
<i>Map length cM</i>		
G_{of}	4134.4	3220.9
G_{oa}	4775.3	4180.2
<i>Estimated map length (cM)</i>		
G_{e1}	5487.6	4538.1
G_{e2}	5514.6	4514.5
G_{e3}	5331.7	4826.1
G_e	5444.6	4626.2
<i>Coverage (%)</i>		
C_{of}	75.9	69.6
C_{oa}	87.7	90.4

Segregation distortion

In the mapping family of this study, segregation distortion was observed for 826 of 2041 AFLP markers (40.5%). When the distorted markers were correlated with the primer combinations, it appeared that a relatively low proportion of distortion markers occurred with certain primer combinations (Table 1). For instance, when primer *MseI*-ACC was used, 25.8% segregated with deviation from Mendelian segregation ratios. Similarly, *MseI*-CGT and *EcoRI*-ACA had relatively low proportions of markers segregating in a non-Mendelian fashion. Microsatellite markers polymorphic in both parents were tested against a 1:1:1:1 (d.f. = 3) segregation ratio. When polymorphic in only one parent, they were tested against a 1:1 (d.f. = 1) segregation. Ten percent of the segregating microsatellite markers were distorted. Deviations from the expected Mendelian ratios have been observed in previous efforts to construct linkage maps using molecular markers. A distortion rate of 65% was reported for clubroot (Voorrips et al. 1997), and 54% was reported for silkworm (Tan et al. 2001) using AFLP markers. The high ratio of segregating markers in the interspecific cross was caused by the distortion of the transmission between genetically divergent genomes (Fishman et al. 2001). Additionally, distorted segregation may also result from the amplification of the same size fragment from several different genomic regions (Faris et al. 1998), competition among gametes for preferential fertilization (Lyttle 1991), genes that are subject to direct selection (Cervera et al. 2001), sampling in finite mapping populations, breaking of DNA chains during extraction (Tan et al. 2001), transposable elements, technical artifacts and so forth.

Sex-linked markers

The sex of the progeny in the mapping family was treated as a marker and was analyzed in both the female and male data. Of the 94 progeny, 54 were female and 40 were male. In the female map, the sex was tightly mapped onto group 29 with seven other markers, including three microsatellite and four AFLP markers (Fig. 1). Three microsatellite markers were linked to sex with 6.6 cM, 8.6 cM and 8.6 cM, and the LOD scores were 17.8, 14.3 and 16.4, respectively. In contrast, there were no markers linked to the sex in the male data.

The confirmation of sex-linked markers on the maternal, but not the paternal map has led to an interesting argument that the female is the heterogametic sex in this shrimp species. With the same

strategy, the sex was also mapped to the female map of the kuruma prawn (Li et al. 2003). Sex determination in the crustacea has been reviewed by Legrand et al. (1987) who noted that the genetic basis for sex determination had been studied in only a few species, none of which were decapods. Shrimp still remains among the list of aquatic crustacean animals in which little is known about sex differentiation and determination. No sex chromosome has been observed in penaeids, nor has any environmental sex determination been reported (Benzie 1998). Although a significant difference in growth rate exists between females and males, as with many other species, an all-female population would increase profitability for shrimp farmers because *L. vannamei* is sexually dimorphic for size at market weight. The current linkage map is the first to map sex-linked markers on the maternal map and to imply that the female may be the heterogametic sex in this shrimp species. We are now testing if these microsatellites are linked with sex in other shrimp families. It has the potential for eventually yielding sex-determining gene sequences in crustaceans.

Future use

In this study, the fluorescence capillary system (ABI 310 and 3100 sequencer) was used to detect PCR products. The advantage of the capillary system over the traditional silver staining is that size standards and sample fragments loaded in the same capillary run undergo the same electrophoretic conditions, which makes the sizing of the AFLPs very precise and genotyping across samples easy and accurate. The accurate sizing of the AFLP and the relative ease of development through the fluorescence capillary system partly compensate for the poor transferability of the AFLP among different labs and populations (Li et al. 2005). Microsatellite markers were approved to be ideal markers for construction of linkage maps because of their high heterozygosity and transferability across different strains within a species, even to different species for those EST-derived markers, there were 18 and 14 microsatellite markers in our sex-specific maps. Based on female and male segregating data, two separate linkage maps were constructed. Theoretically, the two different maps could be merged into a composite map by using markers that are heterozygous in both parents. But such a merge can be poorly performed with dominant markers like AFLPs (Li et al. 2003). In this study, the markers segregating in a 3:1 ratio also showed poor linkage with those segregating in a 1:1 ratio (data not shown), because of only seven common microsatellites in our map, with

more codominant markers such as microsatellite and SNPs mapped to the AFLP-based maps should eventually be merged to one composite map.

This study presents the starting point for further molecular research on *L. vannamei*. The map builds the foundation for thoroughly exploring the entire genome represented by a large number of mapped AFLP and microsatellite markers. It creates a framework for anchoring additional codominant markers such as microsatellite, SNPs and expressed sequence tags, identifying QTL for economically important or evolutionary traits, MAS and comparative genome mapping.

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