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ORIGINAL ARTICLE

Genetic Positioning of Centromeres through Half-Tetrad Analysis in Gynogenetic Diploid Families of the Zhikong Scallop (*Chlamys farreri*)

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Abstract Centromere mapping is a powerful tool for improving linkage maps, investigating crossover events, and understanding chiasma interference during meiosis. Ninety microsatellite markers selected across all linkage groups (LGs) from a previous *Chlamys farreri* genetic map were studied in three artificially induced meiogynogenetic families for centromere mapping by half-tetrad analysis. Inheritance analyses showed that all 90 microsatellite loci conformed to Mendelian inheritance in the control crosses, while 4.4 % of the microsatellite loci showed segregation departures from an expected 1:1 ratio of two homozygote classes in meiogynogenetic progeny. The second division segregation frequency (y) of the microsatellites ranged from 0.033 to 0.778 with a mean of 0.332, confirming the occurrence of partial chiasma interference in this species. Heterogeneity of y is observed in one of 42 cases in which markers were typed in more than one family, suggesting variation in gene–centromere recombination among families. Centromere location was mostly in accordance with the *C. farreri* karyotype, but differences in marker order between linkage and centromere maps occurred. Overall, this study makes the genetic linkage map a more complete and informative tool for genomic studies and it will also facilitate future research of the structure and function of the scallop centromeres.

Keywords *Chlamys farreri* · Microsatellite · Gynogenetic diploid · Half-tetrad analysis · Centromere mapping

Introduction

Gene mapping provides bases for a wide range of genetic and genomic studies, including quantitative trait loci (QTL) identification, marker-assisted selection (MAS), comparative genomics, and evolutionary studies (Danzmann and Gharbi 2001). Genetic linkage maps based on the examination of recombination differences among pairs of genetic markers have been generated in some economically important aquaculture species such as Japanese flounder (Hwang et al. 2011), Nile tilapia (Lee et al. 2011), common carp (Zhang et al. 2011), Atlantic salmon (Li et al. 2011), and striped bass (Liu et al. 2012). In marine mollusks, genetic maps have been established in the Pacific oyster (Hubert and Hedgecock 2004; Li and Guo 2004; Guo et al. 2012), abalone (Sekino and Hara 2007; Zhan et al. 2012), bay scallop (Li et al. 2012), and Zhikong scallop (Zhan et al. 2009). Gene–centromere mapping proved to be an efficient gene mapping technique to facilitate the integration of the centromeric regions in linkage groups (Morishima et al. 2001; Guyomard et al. 2006). Meanwhile, centromere mapping can rapidly provide considerable genetic information about defining fixed points within linkage groups of DNA markers, distinguishing both chromosomal arms, and discovering the interference phenomenon (Bastiaanssen et al. 1996; Park et al. 2007). As all polymorphic genes can be mapped in relation to their centromere, the degree of conservation of gene arrangement among species, and the comparative gene mapping between individuals, populations, and species can be studied (Thorgaard et al. 1983; Allendorf et al. 1986). Thus, centromere mapping is a rational way in providing the basic information about meiosis and exploring the genomic structure for the species of interest.

The principle of centromere mapping is based on half-tetrad analysis, for which two products of second meiotic division can be recovered by inhibiting the release of the second polar body (Zhao and Speed 1998). Meiosis I (MI) nondisjunctions and meiosis II (MII) nondisjunctions are two basic mechanisms that generate half-tetrads as classified by Zhao and Speed (1998). The successful induction of meiogynogenetic diploid or triploid progeny allows MII half-tetrad

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analysis in aquatic organisms in order to estimate the recombination rate between the gene locus and centromere on the chromosome (Lindner et al. 2000; Morishima et al. 2001; Hubert et al. 2009; Li et al. 2009; Nie et al. 2011, 2012). All MII half-tetrad progeny of a heterozygous female will be homozygous if there are no crossovers between the locus and its centromere. A single crossover between a locus and its centromere will create heterozygous progeny (half-tetrads). Thus, the observed proportion of the heterozygotes scored in the mapping panel is taken as a measure of the genetic distance between the centromere and marker as a function of $100\times(y/2)$, where y indicates the proportion of heterozygotes scored (Danzmann and Gharbi 2001).

Half-tetrad analysis is particularly useful for examining meiotic recombination, and it has the flexibility to provide insight into many aspects of inheritance. For example, half-tetrad analysis is a powerful tool for mapping genes and understanding chromosomal behavior during meiosis (Lindner et al. 2000; Hubert et al. 2009). Moreover, half-tetrad analyses are valuable in investigating the crossover events during meiosis because half-tetrads provide information about chromatid interference, chiasma interference, centromere positions, and the order of markers in linkage groups with respect to the centromeres (Johnson et al. 1996; Hubert et al. 2009; Nie et al. 2012). In the last decades, half-tetrad analyses have widely been used for centromere mapping in plant, fish, amphibians, and mammals (e.g., Volpe 1970; Ott et al. 1976; Johnson et al. 1996; Tavoletti et al. 1996). More recently, researchers have also applied this technique to position centromeres on linkage maps in some plants (Park et al. 2007; Okagaki et al. 2008; Cuenca et al. 2011) and several animal species such as rainbow trout (Sakamoto et al. 2000; Guyomard et al. 2006), loach (Morishima et al. 2008), turbot (Martínez et al. 2008), Pacific oyster (Hubert et al. 2009), and Pacific abalone (Nie et al. 2012).

Scallops are one of the most important cultured mollusks in China, with a production of 1,276,770 metric tons in 2009 (DOF 2010). Zhikong scallop (*Chlamys farreri* Jones et Preston 1904) is mainly distributed in coastal areas of north China, Korea, and Japan, and is one of the dominant species of scallop aquaculture. However, mass mortality of this species occurred frequently in many farming areas in China (Zhang and Yang 1999). Today, the mass mortality during summer season is still a major constraint for the development of the scallop culture. In recent years, great progress has been made in scallop genetics and breeding in China to support the sustainable development of scallop farming (Bao et al. 2011). Genetic linkage maps have been generated for *C. farreri* using microsatellite (Zhan et al. 2009) and amplified fragment length polymorphism (AFLP) markers (Wang et al. 2004; Li et al. 2005; Wang et al. 2005). However, no information was available about the positions of centromeres and arm locations of markers on the genetic linkage maps. Therefore, integration of centromeric regions on the map is an issue to be addressed that will make the genetic linkage map a more complete and informative tool for scallop genomic studies.

In the present study, three families of gynogenetic diploid *C. farreri* were produced by inhibition of second polar body. Half-tetrad analyses of these families were carried out for 90 microsatellite DNA markers selected from the scallop genetic map to locate centromere regions in all the linkage groups (LGs) reported by Zhan et al. (2009).

Materials and Methods

Mapping Families and Genomic DNA Extraction

Mature cultured scallops (*C. farreri*) were collected in early May 2008 from the coast of Weihai, Shandong Province, China. Eggs and sperm were obtained by artificially inducing spawning with the stimulation of dryness and raising water temperature. The suspension of sperm and eggs was prepared at a concentration of 1.0×10^7 sperm/ml and 2.0×10^4 egg/ml, respectively. Three families (A, B, and C) were created using eggs and sperm from a single female and male in each case. To produce gynogenetic diploids, eggs were inseminated with ultraviolet (UV)-irradiated sperm followed by inhibition of expulsion of the second polar body with cytochalasin B (0.5 μg/ml; CB) treatment at 25 min post-insemination for 20 min (Pan et al. 2004). The eggs inseminated with normal sperm were used as diploid control. At 40 h after insemination, samples of D-shaped larvae from the treatment and control groups were collected and transferred into 1.5-ml sterile microfuge tubes. In the previous study, the D-shaped larvae which did not occur in the haploid group but were observed in the CB-treated gynogenetic diploid groups were confirmed to be gynogenetic diploids (Li et al. 2009). After gentle centrifugation at $800\times g$ for 5 min, seawater was removed and larvae were then preserved in 100 % ethanol at 4 °C.

Genomic DNA was extracted from adductor muscle of parental scallops and whole D-shaped larvae. DNA was isolated from adductor muscle of the individual broodstock using a standard phenol/chloroform extraction procedure according to Li et al. (2002). Larval DNA was prepared by the Chelex modification extraction method as previously described by Li et al. (2003).

Microsatellite Genotyping

A total of 137 microsatellites mapped on the scallop linkage maps (Zhan et al. 2009) were evaluated for mapping suitability through polymerase chain reaction (PCR) amplification and genotyping of parental DNA, and 90 markers turned out to be heterozygous in at least one of the three mapping families. PCR was performed in a 10-μl volume containing 0.25 U *Taq*

DNA polymerase (Takara), 1× PCR buffer, 0.2 mM dNTPs, 1 μM of each primer set, 1.5 mM $MgCl_2$, and 1 μl of total DNA extracted from adults or larvae. PCR was performed on a GeneAmp 9700 PCR system (Applied Biosystems) with cycling parameters as follows: 3 min at 94 °C; seven cycles of 1 min at 94 °C, 30 s at the optimal annealing temperature, and 30 s at 72 °C; 33 cycles of 30 s at 94 °C, 30 s at annealing temperature, 30 s at 72 °C, with a final extension of 5 min at 72 °C. Amplification products were resolved on a 6 % denaturing polyacrylamide gel and visualized by silver staining.

Marker–Centromere Recombination Frequency

According to the half-tetrad analysis method (Thorgaard et al. 1983; Zhao and Speed 1998; Nie et al. 2012), marker–centromere (M–C) recombination rates that were revealed by the second division segregating frequency (y) were estimated as the frequency of the heterozygous recombinant genotype in the meiotic gynogenetic progeny. The map distance in centimorgans (cM) in relation to the centromere will equal to $100\times(y/2)$ under the assumption of complete interference (Thorgaard et al. 1983). Since null homozygotes cannot be distinguished from PCR failure, markers for which the maternal parent was a null heterozygote were discarded. Heterogeneity of y between families for markers segregated in two or three families was tested using a contingency χ^2 test before pooling the data for each locus. To ascertain if alleles are inherited in a Mendelian fashion, all observed progeny ratios of each primer set were tested in control groups against the expected Mendelian segregation ratios (1:1, 1:2:1, and 1:1:1:1) using chi-square analysis ($P<0.05$) (with $n-1°$ of freedom, where n = number of phenotypic classes). Corrections of the significance level for multiple tests were performed following the sequential Bonferroni procedure (Rice 1989).

Centromere Mapping in Scallop Linkage Groups

The integrated linkage map of Zhikong scallop reported by Zhan et al. (2009) was set as a framework to locate centromeres. Centromeres were mapped through half-tetrad analysis for several loci per linkage group by genotyping three meiogynogenetic diploid families which consisted of 60 individuals, respectively. For each linkage group, two markers located at two opposite ends of each linkage group were initially selected to ascertain centromere orientation along the chromosomal axis. When large M–C distances were observed with both terminal markers, the centromere was considered to be located at an internal position between the two markers. Additional markers were then selected for a more precise location (Nie et al. 2012). The order of markers in each LG was compared with that reported by Zhan et al. (2009), and a centromere linkage map was created based on distance of each marker from the centromere, as described by Johnson et al. (1996). The 95 % confidence interval around centromere markers for probable centromere location was calculated according to the formula $y/N\pm1.96\{[(y/N)(1-y/N)]/N\}^{1/2}$, where y represents the number of heterozygous progeny for the indicated locus, N is twice the number of progeny (Johnson et al. 1996).

Results

Mendelian Segregation

Genotypic segregation of 137 microsatellite loci was examined in three full-sib families. Ninety microsatellite loci which were heterozygous in the female parents were used to evaluate the inheritance in three meiogynogenetic families and their control groups (Appendix). Of the 145 genotypic ratios examined, the offspring genotypes in 111 segregations conform to Mendelian inheritance at the 5 % level after sequential Bonferroni correction for multiple tests. The remaining 34 genotypic ratios at 30 loci were in accordance with Mendelian expectations when we assumed that the male parent carried one or two null alleles, and unexpected offspring genotypes were homozygotes and heterozygotes for null alleles.

Verification of Meiotic Gynogenesis

Both parents and 60 meiogynogenetic diploid progeny of each family were genotyped for diagnostic microsatellites to confirm their meiogynogenetic diploid constitution and the exclusive maternal inheritance, as previously reported by Li et al. (2009). The male parent carried 48 unique alleles at 36 loci in family A, 53 unique alleles at 39 loci in family B, and 40 unique alleles at 30 loci in family C (Appendix). The unique alleles were absent in the counterpart female and thus were useful to examine whether male parent contributed to the genomes of the offspring. No unique paternal allele was found in any of the offspring of any of the gynogenetic groups (N=7,572), which confirmed the 100 % success of induction of gynogenesis. None of the 40-h D-shaped larvae sampled were homozygous at all loci examined. This indicated that these larvae were not haploids and were true gynogenetic diploids that could be used for centromere mapping.

M–C Recombination Frequency

The three scallop females used to produce gynogenetic diploids were heterozygous for 55, 48, and 42 microsatellite loci at families A, B, and C, respectively, allowing M–C distance estimation for these loci (Appendix). The number of progeny in two homozygote classes was compared with an expected 1:1 ratio, most cases of which met the expectation. However,

four cases showed segregation distortion at the 5 % level after Bonferroni correction, as would be expected if one of the homozygotes had reduced viability because of homozygosity for the locus itself or linkage to a recessive deleterious allele (Appendix). These cases were eliminated from calculations of marker–centromere distances because unequal proportions of homozygotes suggest selection against a viability locus linked to one of the alleles, which could affect estimation of M–C distance. One of the four cases was genotyped in only one family (CFKD102 in family A), and this marker was excluded from further analyses. Two families were typed for the same marker in 29 cases, and only one of them (CFMSM014 in family A and B) showed heterogeneity of y by contingency χ^2 test ($P<0.01$) (Appendix). Three families were typed for the same marker in 13 cases, and none of them showed heterogeneity of y among families by contingency χ^2 test ($P<0.01$).

Genotypes of 90 microsatellite loci examined in three gynogenetic families revealed that the proportion of heterozygotes (y) ranged from 0.033 at CFLD006 to 0.778 at CFFD163, with an average of 0.332. The heterozygotes frequencies (y) for the 90 microsatellite loci are non-uniformly distributed over the interval from 0.0 to 1.0 ($\chi^2=87.4$, 9 *df*, $P<0.001$; Fig. 1). The numbers of microsatellites distributed among the five arbitrarily divided regions of chromosome based on the y values (0–0.19, 0.20–0.39, 0.40–0.59, 0.60–0.79, and 0.80–1.00) were 22 (24.4 %), 35 (38.9 %), 25 (27.8 %), 8 (8.9 %), and 0 (0 %) respectively (Fig. 1). High M–C recombination frequencies were obtained at CFMSM014 (0.714) and CFFD163 (0.778), whereas low M–C recombination frequencies were observed at six loci ($y<0.1$). The other 82 microsatellite loci showed intermediate y values between 0.103 (CFFD093) and 0.632 (CFZB112 and CFLD060). Four loci, CFLD006, CFAD019, CFBD169, and CFBD217, were closely linked to their centromeres as indicated by M–C distances of 1.65, 2.00, 2.08, and 2.17 cM, respectively. In the three gynogenetic families, two loci scored y greater than 0.67, a value which is expected for markers segregating independently with respect to centromeres (Mather 1935; Thorgaard et al. 1983), indicating the existence of partial interference after a single chiasma formation in some chromosomes. Only two (2.2 %) of 90 markers with y value exceeding 0.67 distributed in two LGs (CFFD163 in LG1 and CFMSM014 in LG16) were observed, which implies that chiasma interference did not frequently occur in *C. farreri* chromosomes.

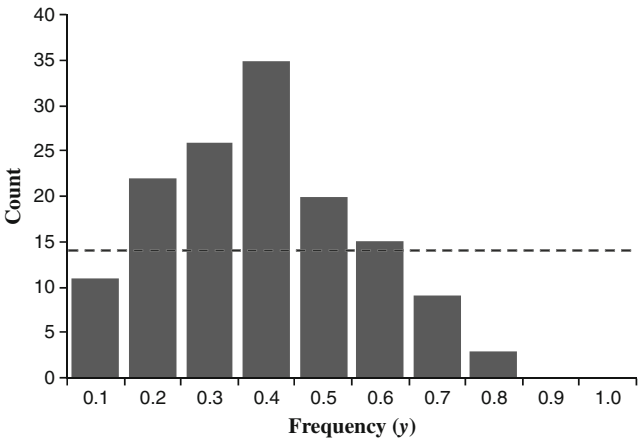

Fig. 1 The distribution of heterozygotes frequencies (y) for the 90 microsatellites estimated in three meiogynogenetic diploid families. *Dotted line* gives the expectation for the uniform distribution, from which the observations significantly differ ($\chi^2=87.4$, 9 *df*, $P<0.001$)

Positioning the Centromere

The genetic linkage map for the *C. farreri* (Zhan et al. 2009) was used to assign 90 markers to linkage groups (LGs), resulting in two to eight loci per LG in the centromere map with an average of four to five loci per LG. These were ordered by marker–centromere distances to produce a centromere linkage map (Fig. 2), where the short arm of a chromosome (LG) is placed upward designating the position of the most distal marker (from centromere) on the short arm as position "zero". The position of the centromere was estimated as the region indicated by the 95 % confidence intervals inferred from the marker near the centromere under the assumption of complete interference. Markers not located near the centromere can be ordered relative to each other and the centromere by half-tetrad analysis (Johnson et al. 1996). We identified 11 microsatellite loci (CFFD163 in LG1; CFZB112 in LG3; CFJD036 and CFFD031 in LG4; CFFD144 and CFAD104 in LG5; CFFD110 in LG7; CFFD067 and CFBD159 in LG8; CFLD009 in LG11; CFBD222 in LG14) (Appendix) that showed apparent discrepancy between the centromere map and the published map (Zhan et al. 2009). As shown in Fig. 2, four LGs (LG2, LG6, LG8, and LG17) were estimated to map the centromere at an intermediate position on the chromosome, whereas six LGs (LG1, LG3, LG9, LG12, LG13, and LG15) had the centromere located at the sub-intermediate position between the short arm and long arms of the chromosomes, and the other nine LGs were estimated to be acrocentric or telocentric chromosomes.

Discussion

Segregation Distortion

Growing evidence indicates that widespread presence of null alleles and a high ratio of segregation distortion seem to be two characteristics of marine molluscs (Launey and Hedgecock 2001; Zhan et al. 2009). In the present study, however, we found no evidence of segregation distortion in the D-larvae from the control crosses based on the fact that all of the 90 microsatellite loci (145 tests) segregated as expected in a Mendelian fashion, suggesting that segregation analysis was performed in the 40-

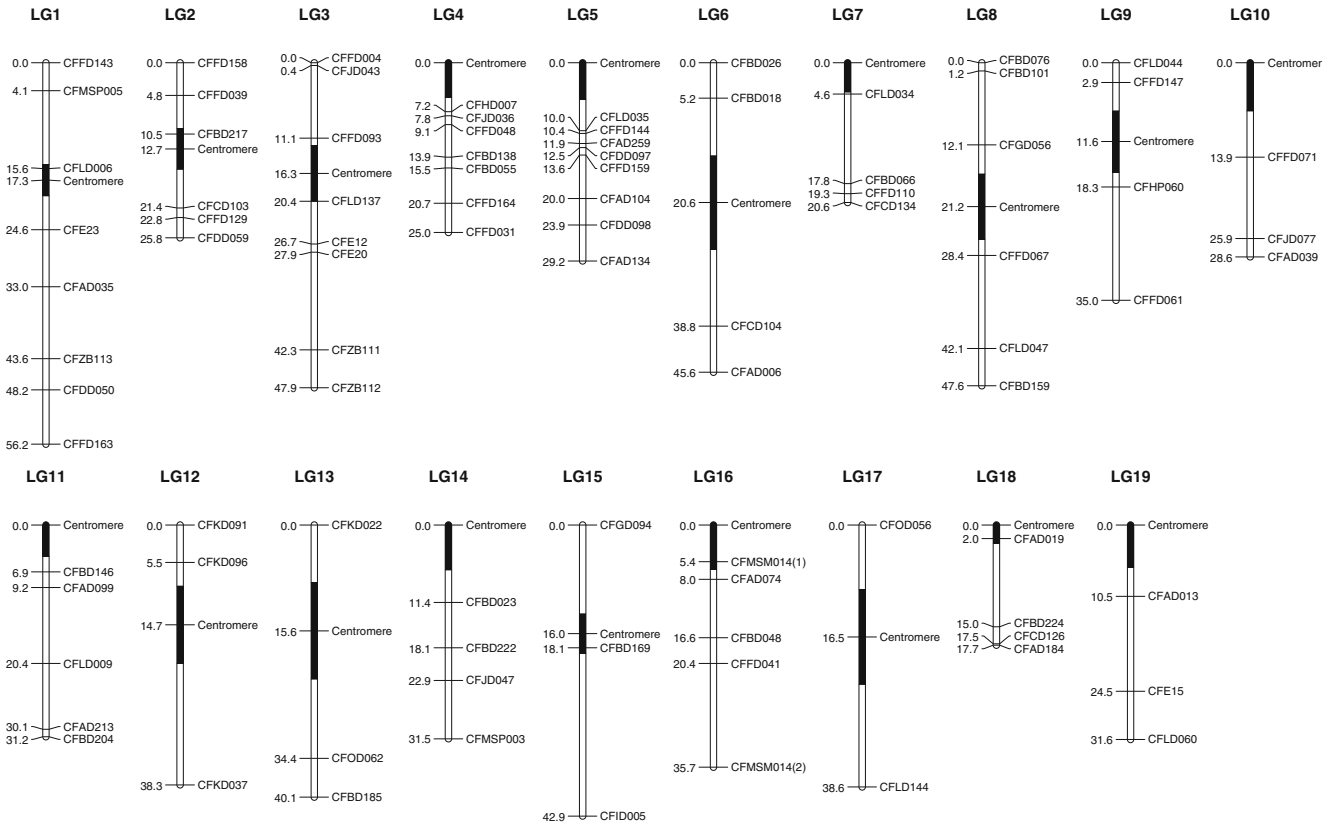

Fig. 2 Centromere linkage map with corrected orientation and centromeric positions in the *C. farreri* after half-tetrad segregation analysis. Centromere locations determined by half-tetrad analysis are indicated by *black rectangles* (95 % confidence interval)

h D-larvae before defective genes were turned on and expressed. This is consistent with previous studies which found generally undistorted allele frequencies in invertebrate early larval stage, while adults had allele frequencies which strongly departed from expected frequencies (Launey and Hedgecock 2001; Nie et al. 2011). Therefore, genotyping marine invertebrate larvae is a useful strategy for gene mapping to reduce or eliminate segregation distortion caused by selection (Hubert and Hedgecock 2004; Foley et al. 2011).

Recessive lethal or deleterious genes may cause a significant segregation distortion in diploid meiogynogenetic families (Allendorf et al. 1986). The meiogynogens genomes are likely to carry a number of alleles associated with differential survival of the two homozygous classes (Martínez et al. 2008). In this study, four of the 90 loci (4.4 %) showed segregation distortions from an expected 1:1 ratio of two homozygote classes in the diploid gynogenetic progeny. Because alleles at the microsatellite locus segregated at the expected Mendelian ratio in the control crosses using the same female, the segregation departure might result from homozygous of recessive lethal genes caused by the induction of gynogenesis. Meiogynogenetic diploids might carry deleterious alleles that would negatively affect their survival, and this is common in shellfish and fish (Hubert et al. 2009). Because the causes of non-1:1 homozygote ratios could potentially affect the proportion of heterozygotes, *y*, and thus the distance of the marker from the centromere, we eliminated these cases for further analyses.

Null Alleles in Microsatellite Loci

The widespread presence of null alleles at microsatellite loci is not uncommon in marine bivalves (Hedgecock et al. 2004; Zhan et al. 2009). For example, 51 % of the microsatellite loci contain at least one null allele detected in three mapping families of the *Crassostrea gigas* (Hubert and Hedgecock 2004). It is known that null allele is a potential drawback in genetic analyses, which can lead to negative effects on linkage map construction (Zhan et al. 2009). Null alleles of microsatellite regions, which occasionally fail to yield an amplification product, may arise through mutations such as single nucleotide mutations in the primer annealing site (Callen et al. 1993; Pemberton et al. 1995). In *C. farreri*, Zhan et al. (2009) also reported a comparatively high ratio (56.2 %) of null alleles. In this study, 30 of the 90 microsatellite loci (33.3 %) have null allele problems in three families of the Zhikong scallop. These null alleles were confirmed by the fact that a progeny with a single locus genotype of A/A was known to be the offspring of parents with genotypes A/B and C/C, and the parent with genotype C/C was concluded to have a null allele (C/null). Because homozygous for a null allele cannot be distinguished

from PCR failure, markers for which the maternal parent was a null heterozygote were eliminated. In population studies, the presence of null alleles may complicate the interpretation of deviations from Hardy–Weinberg equilibrium, and multiple nulls could confuse analyses of population structure.

M–C Recombination Frequency

The merit of using multiple families for map construction is not only to increase the map density of consolidated maps but also to allow assessment of heterogeneities in the recombination rate between families (Sekino and Hara 2007). For most of the 29 markers typed in two families and all the 13 markers typed in three families, similar y values were obtained in different families, suggesting the reproducibility of the M–C distance estimation. However, differences in genotypic ratios of offspring between gynogenetic families were significant at one locus (CFMSM014 in family A and B). Variation in recombination rates among different families was unveiled in the Pacific abalone, which is caused most probably by chromosomal variations (Sekino and Hara 2007). Extensive differences in recombination rate within sexes have also been observed in *C. gigas* and were ascribed to the possible existence of polymorphism for chromosomal rearrangements (Hubert and Hedgecock 2004; Hubert et al. 2009). The possible cause for the differential genotypic ratio among gynogenetic families of *C. farreri* in this work might be associated with the effects that gynogenesis, and the inbreeding it generates, has on larval survival. Chromosomal variations could also account for the differences in recombination rate among individuals (Liu 1998). More dense linkage and centromere maps are needed to confirm whether the Zhikong scallop is polymorphic for chromosomal rearrangements.

The M–C recombination frequency for the 90 microsatellite loci was low, averaging 0.33, suggesting that the distribution of crossovers is not random, but biased toward the telomeric region. Random distribution of crossovers would produce an average gene–centromere recombination frequency of 0.50 (Guo et al. 2008). Recombination frequencies observed in this study were frequently lower than the theoretical maximum of 0.67 expected from independent recombination events and appeared skewed toward low values, indicating that crossover interference is not commonly existent in the scallop chromosomes. In contrast, higher M–C recombination frequencies ($y>$ 0.67) have been recorded in a wide variety of fish species (Danzmann and Gharbi 2001 and references therein) and are generally attributed to strong chiasma interference occurring after the formation of a single chiasma. The average recombination rate of *C. farreri* (0.33) is relatively low compared to other aquaculture species studied, such as large yellow croaker (0.59), Chinese shrimp (0.47), and Pacific abalone (0.40) (Li et al. 2008; Wang et al. 2008; Nie et al. 2012), suggesting that most markers are close to centromeres or alternatively that single crossing-over is low between homologues in this species. To properly evaluate the low recombination rate during meiosis in *C. farreri*, further information on chromosome size and map position of the markers is needed.

Fixation Index

Gene–centromere recombination rates in bivalve molluscs have been estimated from autotriploids (Hubert et al. 2009), but most estimates had been performed utilizing gynogenetic-induced progeny (Guo and Allen 1996; Li and Kijima 2005, 2006; Li et al. 2009; Nie et al. 2012). Microsatellite analysis showed that all the gynogenetic diploid progeny possessed only the alleles of their mothers, confirming exclusive maternal inheritance. The 100 % success demonstrates that optimum conditions of UV irradiation for complete genetic inactivation of *C. farreri* sperm were obtained in this study. The rate of inbreeding can be estimated by the fixation index (F) (Allendorf et al. 1986), which is calculated simply by $F=1-y$ in a one-generation gynogenetic family (Allendorf and Leary 1984). The estimation of mean y (0.33) corresponds to an F of 0.67, which is 2.7 times higher than the inbreeding coefficient after one generation of sib-mating ($F=0.25$) or 1.3 times higher than self-fertilizing ($F=0.5$) (Purdom 1983; Allendorf et al. 1986). Thus, meiotic gynogenesis could provide an effective means of rapid inbreeding in the scallop. Although genes distantly located from the centromere (large y) will retain heterozygosity in ensuing generations, repeated meiotic gynogenesis may be a practical way to procure inbred lines in *C. farreri*.

Chiasma Interference

Estimated M–C recombination rates ranged between 0.033 and 0.778. Thus, map distance under complete interference varied between 1.7 and 38.9 cM. This result demonstrates that the microsatellite loci widely distribute from proximal (centromeric) to distal (telomeric) regions of scallop chromosomes. A marker unlinked to its centromere has a half-tetrad recombinant fraction y of 2/3 (0.67). Preference for an odd number of crossover (probably single) results in $y>2/3$; this is called “chiasma interference”. In aquatic animals, very high levels of chiasma interference have been reported in some fish revealed by M–C recombination fractions that ranged as high as 100 %, such as salmonids (Thorgaard et al. 1983; Allendorf et al. 1986) and channel catfish (Liu et al. 1992), and it has been suggested that the phenomenon might be general for fish. However, as for most shellfish species, gene–centromere mapping revealed a few loci with $y>0.67$ and relatively low mean y values, such as Zhikong scallop (0.41; Li et al. 2009), Pacific oyster (0.41; Hubert et al. 2009), and Pacific abalone (0.40; Nie et al. 2012), suggesting low or moderate levels of chiasma interference. Whereas in the dwarf-surf clam *Mulinia lateralis*, Guo and Allen (1996)

surprisingly found that the gene–centromere recombination rate is 100 % for most allozyme loci, suggesting that there is always one and only one crossover occurring close to the centromere. In this study, values of y >0.67 shown at the two of the 90 microsatellites (CFMSM014 and CFFD163; 2.2 %) indicate the existence of positive interference after a single chiasma formation in some scallop chromosomes. That is, one crossover most likely partially inhibited the occurrence of another crossover in the same interval on the chromosome arm carrying the CFMSM014 and CFFD163 locus. Only two loci with y >0.67 obtained from the results of half-tetrad analysis implies that chiasma interference did not frequently occur in *C. farreri* chromosomes.

Centromere Mapping

Centromeres are the complex chromosomal structures responsible for proper eukaryotic chromosome segregation in meiosis and mitosis by nucleating kinetochore formation, providing the site for microtubule attachment, and maintaining sister chromatid cohesion. In addition, centromeres also have an important role in checkpoint regulation during mitosis (Pluta et al. 1995). Identification of the genetic position of centromeres, which is important for distinguishing chromosome arms and identifying proximal and distal markers or genes, is the first step towards understanding the composition and structure of the centromeric region (Bastiaanssen et al. 1996; Park et al. 2007). Mapping of centromeric regions on the linkage map has been reported in several plants (Park et al. 2007; Okagaki et al. 2008; Cuenca et al. 2011) and animals (Sakamoto et al. 2000; Guyomard et al. 2006; Morishima et al. 2008; Martínez et al. 2008; Hubert et al. 2009; Nie et al. 2012). In this study, we inferred the position of the centromeric region for each of the 19 chromosomes using microsatellite markers on the linkage map constructed by Zhan et al. (2009). From the results of the centromere mapping, four LGs (LG2, LG6, LG8, and LG17) were estimated to map the centromere at an intermediate position on the chromosome, whereas six LGs (LG1, LG3, LG9, LG12, LG13, and LG15) had the centromere located at the sub-intermediate position between the short arm and long arms of the chromosomes. In *C. farreri*, the diploid karyotype was reported to comprise four pairs of metacentric, six pairs of submetacentric, and nine pairs of acrocentric or telocentric chromosomes (Komaru and Wada 1985). Thus, the number of LGs that have the centromere at the intermediate or sub-intermediate position corresponds well with the number of the bi-arm chromosomes in the scallop karyotype. Although mapping of the centromere regions on all linkage groups have been accomplished by half-tetrad analysis, present centromere maps of microsatellites for the Zhikong scallop are revealed as incomplete.

Overall, marker orders and distances among markers estimated by half-tetrad analyses are similar to those obtained in the previous linkage map (Zhan et al. 2009). However, 11 of 90 markers showed an obvious discrepancy. The occurrence of these aberrant markers could be attributable to differences in recombination frequency in different sex (Sakamoto et al. 2000; Hubert and Hedgecock 2004; Sekino and Hara 2007; Zhan et al. 2009). Higher recombination rates in females than in males have been reported in several molluscan species including oyster (Yu and Guo 2003; Li and Guo 2004), abalone (Liu et al. 2006), and the scallop (Zhan et al. 2009). An integrated map constructed by Zhan et al. (2009) was adopted as a framework in this study while the recombination data obtained here was from gynogenetic families. Hubert and Hedgecock (2004) reported that recombination rates and even gene orders can be significantly different between full siblings of *C. gigas* that are derived from the natural population. This suggests that polymorphism for chromosomal rearrangements in the natural populations may be an alternative explanation for the differences in marker order.

The position of the centromere in all the LGs of the Zhikong scallop was inferred in this study, which enhances our understanding of the recombination mode along with the chromosomes in *C. farreri*. Centromere markers, in combination with half-tetrad analyses in induced gynogenetic families, are a valuable genomic resource for the scallop because they permit the rapid assignment of new markers to a linkage group (Johnson et al. 1996; Hubert et al. 2009). Localization of centromeric regions on the genetic linkage map makes the map a more complete and informative tool for genomic studies, and it will also facilitate future study of the structure and function of the scallop centromeres.

Conclusions

Heterozygote frequencies of the 90 microsatellites ranged from 0.033 to 0.778 with a mean of 0.332, indicating the existence of interference in *C. farreri* chromosomes. Centromeres were mapped for all the linkage groups through half-tetrad analysis in meiogynogenetic diploid families. Integration of a microsatellite–centromere map and linkage map will satisfy an essential requisite not only in elucidating syntenies among different species but also in identifying commercially important quantitative traits in the aquaculture species. Nevertheless, extending the coverage of the genome with an increased number of markers is needed to verify the centromere positions inferred in this study. High-density linkage maps with the location information of the centromere will facilitate comparative mapping, identification of candidate genes in QTL studies, and, eventually, apply MAS to genetic improvement of scallop strains for aquaculture.

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Appendix

Table 1 Genotypic ratios of 90 microsatellite loci in gynogenetic and control families of *C. farreri*

Linkage group	Locus	Family	Parental genotypes	Genotypes of control progeny					Genotypes of gynogenetic progeny			χ^2	*P* value	*y*
LG1	CFFD143	A	♀ A/B ♂ A/C	A/A 8	A/C 8	A/B 5	B/C 11		A/A 16	A/B 20	B/B 20	2.250	0.522	0.357
		C	♀ A/B ♂ C/C	A/C 13	B/C 19				A/A 26	A/B 20	B/B 14	1.125	0.289	0.333
	CFMSP005	C	♀ A/B ♂ A/B	A/A 8	A/B 13	B/B 9			A/A 20	A/B 15	B/B 22	0.600	0.741	0.263
	CFLD006	B	♀ A/B ♂ C/D	A/C 9	A/D 11	B/C 6	B/D 4		A/A 34	A/B 2	B/B 24	3.867	0.276	0.033
	CFE23	A	♀ A/B ♂ A/C	A/A 9	A/C 8	A/B 7	B/C 4		A/A 32	A/B 8	B/B 16	2.000	0.572	0.143
		B	♀ A/B ♂ C/C	A/C 15	B/C 16				A/A 26	A/B 4	B/B 24	0.032	0.857	0.074
		C	♀ A/B ♂ C/D	A/C 3	A/D 13	B/C 4	B/D 12		A/A 20	A/B 12	B/B 22	10.250	0.017	0.222
	CFAD035	A	♀ A/B ♂ B/C	A/B 11	A/C 10	B/B 8	B/C 1		A/A 18	A/B 15	B/B 15	8.133	0.043	0.313
	CFKD102	A	♀ A/B ♂ C/D	A/C 8	A/D 11	B/C 5	B/D 5		A/A 26	A/B 22	B/B 4	3.414	0.332	**0.423**
	CFZB113	C	♀ A/B ♂ A/B	A/A 10	A/B 13	B/B 8			A/A 15	A/B 30	B/B 12	1.065	0.587	0.526
	CFDD050	A	♀ A/B ♂ A/null	A/B 10	B/null 9	A/(A or null) 12			A/A 16	A/B 32	B/B 8	1.645	0.439	0.571
		B	♀ A/B ♂ C/C	A/C 16	B/C 16				A/A 5	A/B 32	B/B 13	0.000	1.000	0.650
		C	♀ A/B ♂ A/C	A/A 6	A/C 8	A/B 6	B/C 11		A/A 5	A/B 30	B/B 13	2.161	0.540	0.632
	CFFD163	B	♀ A/B ♂ C/C	A/C 15	B/C 15				A/A 5	A/B 35	B/B 5	0.000	1.000	0.778
LG2	CFFD158	A	♀ A/B ♂ A/C	A/A 9	A/C 7	A/B 6	B/C 9		A/A 14	A/B 8	B/B 24	0.871	0.832	0.174
		C	♀ A/B ♂ C/null	A/C 4	B/C 8	A/null 8	B/null 11		A/A 12	A/B 15	B/B 18	3.194	0.363	0.333
	CFFD039	A	♀ A/B ♂ C/D	A/C 8	A/D 4	B/C 14	B/D 4		A/A 20	A/B 6	B/B 26	8.933	0.030	0.115
		C	♀ A/B ♂ C/null	A/C 9	B/C 3	A/null 8	B/null 11		A/A 27	A/B 12	B/B 21	4.484	0.214	0.200
	CFBD217	B	♀ A/B ♂ C/null	A/C 11	B/C 8	A/null 5	B/null 8		A/A 28	A/B 2	B/B 16	2.250	0.522	0.043
	CFCD103	A	♀ A/B ♂ B/B	A/B 15	B/B 17				A/A 15	A/B 9	B/B 24	0.125	0.724	0.188
		B	♀ A/B ♂ C/C	A/C 19	B/C 13				A/A 27	A/B 9	B/B 21	1.125	0.289	0.158
	CFFD129	A	♀ A/B ♂ A/C	A/A 6	A/C 6	A/B 9	B/C 6		A/A 18	A/B 18	B/B 22	1.000	0.801	0.310
		B	♀ A/B ♂ C/D	A/C 8	A/D 3	B/C 10	B/D 11		A/A 23	A/B 5	B/B 25	4.750	0.191	0.094
	CFDD059	A	♀ A/B ♂ A/A	A/A 18	A/B 12				A/A 16	A/B 12	B/B 18	1.200	0.273	0.261
LG3	CFFD004	B	♀ A/B ♂ C/D	A/C 7	A/D 10	B/C 5	B/D 7		A/A 12	A/B 16	B/B 20	1.759	0.624	0.333

Table 1 (continued)

Linkage group	Locus	Family	Parental genotypes	Genotypes of control progeny					Genotypes of gynogenetic progeny			χ^2	*P* value	*y*
		C	♀ A/B ♂ B/null	A/B 5	A/null 8	B/(B or null) 17			A/A 12	A/B 14	B/B 18	1.133	0.567	0.318
	CFJD043	A	♀ A/B ♂ B/C	A/B 9	A/C 10	B/B 8	B/C 1		A/A 36	A/B 12	B/B 12	7.143	0.067	**0.200**
		C	♀ A/B ♂ B/null	A/B 3	A/null 11	B/(B or null) 16			A/A 12	A/B 14	B/B 18	4.400	0.111	0.318
	CFFD093	A	♀ A/B ♂ B/B	A/B 17	B/B 13				A/A 30	A/B 6	B/B 22	0.533	0.465	0.103
	CFLD137	A	♀ A/B ♂ C/D	A/C 7	A/D 13	B/C 7	B/D 4		A/A 18	A/B 6	B/B 28	5.516	0.138	0.115
		B	♀ A/B ♂ A/C	A/A 9	A/C 4	A/B 4	B/C 7		A/A 14	A/B 2	B/B 26	3.000	0.392	0.048
	CFE12	A	♀ A/B ♂ B/C	A/B 6	A/C 5	B/B 12	B/C 9		A/A 24	A/B 8	B/B 20	3.750	0.290	0.154
		C	♀ A/B ♂ A/C	A/A 5	A/C 3	A/B 4	B/C 6		A/A 21	A/B 15	B/B 21	1.250	0.741	0.263
	CFE20	A	♀ A/B ♂ B/B	A/B 11	B/B 17				A/A 27	A/B 12	B/B 17	1.286	0.257	0.214
		C	♀ A/B ♂ A/B	A/A 6	A/B 13	B/B 12			A/A 14	A/B 12	B/B 22	3.129	0.209	0.250
	CFZB111	A	♀ A/B ♂ A/C	A/A 15	A/C 3	A/B 6	B/C 7		A/A 12	A/B 25	B/B 20	10.161	0.017	0.438
		C	♀ A/B ♂ C/null	A/C 4	B/C 11	A/null 7	B/null 10		A/A 15	A/B 36	B/B 9	3.750	0.290	0.600
	CFZB112	B	♀ A/B ♂ A/null	A/B 7	B/null 6	A/(A or null) 18			A/A 6	A/B 36	B/B 15	0.871	0.647	0.632
LG4	CFHD007	B	♀ A/B ♂ A/null	A/B 10	B/null 5	A/(A or null) 15			A/A 24	A/B 9	B/B 15	1.667	0.435	0.188
		C	♀ A/B ♂ B/C	A/B 7	A/C 9	B/B 6	B/C 9		A/A 17	A/B 5	B/B 28	0.871	0.832	0.100
	CFJD036	B	♀ A/B ♂ C/C	A/C 15	B/C 17				A/A 20	A/B 2	B/B 20	0.125	0.724	0.048
		C	♀ A/B ♂ C/C	A/C 16	B/C 16				A/A 26	A/B 15	B/B 16	0.000	1.000	0.263
	CFFD048	B	♀ A/B ♂ C/D	A/C 8	A/D 5	B/C 14	B/D 5		A/A 25	A/B 10	B/B 20	6.750	0.080	0.182
	CFBD138	A	♀ A/B ♂ A/null	A/B 12	B/null 7	A/(A or null) 13			A/A 12	A/B 15	B/B 27	2.688	0.261	0.278
	CFBD055	B	♀ A/B ♂ C/D	A/C 10	A/D 8	B/C 7	B/D 7		A/A 15	A/B 12	B/B 25	0.750	0.861	0.231
		C	♀ A/B ♂ B/B	A/B 13	B/B 19				A/A 15	A/B 21	B/B 18	1.125	0.289	0.389
	CFFD164	A	♀ A/B ♂ A/null	A/B 4	B/null 14	A/(A or null) 12			A/A 16	A/B 24	B/B 18	7.867	0.020	0.414
	CFFD031	A	♀ A/B ♂ A/B	A/A 12	A/B 11	B/B 8			A/A 18	A/B 24	B/B 6	3.645	0.162	0.500
LG5	CFLD035	C	♀ A/B ♂ C/null	A/C 5	B/C 8	A/null 10	B/null 8		A/A 20	A/B 12	B/B 28	1.645	0.649	0.200
	CFFD144	B	♀ A/B ♂ C/D	A/C 11	A/D 14	B/C 4	B/D 2		A/A 24	A/B 10	B/B 14	12.484	0.006	0.208
	CFAD259	B	♀ A/B ♂ C/null	A/C 10	B/C 8	A/null 9	B/null 5		A/A 22	A/B 18	B/B 12	1.750	0.626	0.346
		C	♀ A/B ♂ B/C	A/B 4	A/C 12	B/B 7	B/C 7		A/A 25	A/B 6	B/B 15	4.400	0.221	0.130

Table 1 (continued)

Linkage group	Locus	Family	Parental genotypes	Genotypes of control progeny					Genotypes of gynogenetic progeny			χ^2	*P* value	*y*
	CFDD097	C	♀ A/B ♂ C/D	A/C 8	A/D 8	B/C 10	B/D 6		A/A 23	A/B 12	B/B 13	1.000	0.801	0.250
	CFFD159	A	♀ A/B ♂ C/D	A/C 8	A/D 4	B/C 8	B/D 7		A/A 22	A/B 18	B/B 16	1.593	0.661	0.321
		B	♀ A/B ♂ B/B	A/B 14	B/B 18				A/A 20	A/B 12	B/B 22	0.500	0.480	0.222
	CFAD104	B	♀ A/B ♂ B/C	A/B 8	A/C 5	B/B 11	B/C 7		A/A 14	A/B 20	B/B 16	2.419	0.490	0.400
	CFDD098	B	♀ A/B ♂ C/null	A/C 4	B/C 5	A/null 6	B/null 12		A/A 14	A/B 22	B/B 10	5.741	0.125	0.478
	CFAD134	C	♀ A/B ♂ A/B	A/A 3	A/B 23	B/B 5			A/A 9	A/B 35	B/B 16	7.516	0.023	0.583
LG6	CFBD026	B	♀ A/B ♂ C/null	A/C 5	B/C 10	A/null 9	B/null 8		A/A 20	A/B 21	B/B 10	1.750	0.626	0.412
	CFBD018	B	♀ A/B ♂ C/D	A/C 5	A/D 8	B/C 8	B/D 8		A/A 23	A/B 16	B/B 13	0.931	0.818	0.308
	CFCD104	A	♀ A/B ♂ A/B	A/A 0	A/B 24	B/B 8			A/A 15	A/B 20	B/B 20	12.000	0.002	0.364
	CFAD006	A	♀ A/B ♂ C/null	A/C 7	B/C 7	A/null 5	B/null 13		A/A 10	A/B 28	B/B 18	4.500	0.212	0.500
LG7	CFLD034	A	♀ A/B ♂ A/A	A/A 16	A/B 14				A/A 12	A/B 4	B/B 28	0.143	0.705	0.091
	CFBD066	A	♀ A/B ♂ C/null	A/C 8	B/C 3	A/null 9	B/null 12		A/A 18	A/B 21	B/B 21	5.250	0.154	0.350
	CFFD110	B	♀ A/B ♂ A/C	A/A 6	A/C 6	A/B 14	B/C 6		A/A 21	A/B 20	B/B 11	6.000	0.112	0.385
	CFCD134	A	♀ A/B ♂ A/B	A/A 9	A/B 11	B/B 12			A/A 25	A/B 24	B/B 5	3.688	0.158	**0.444**
		B	♀ A/B ♂ A/B	A/A 7	A/B 13	B/B 7			A/A 10	A/B 22	B/B 14	0.037	0.982	0.478
		C	♀ A/B ♂ C/null	A/C 9	B/C 4	A/null 10	B/null 9		A/A 12	A/B 18	B/B 22	2.750	0.432	0.346
LG8	CFBD076	A	♀ A/B ♂ B/C	A/B 10	A/C 10	B/B 3	B/C 7		A/A 21	A/B 15	B/B 18	4.400	0.221	0.278
		C	♀ A/B ♂ A/C	A/A 12	A/C 5	A/B 6	B/C 9		A/A 16	A/B 32	B/B 8	3.750	0.290	0.571
	CFBD101	A	♀ A/B ♂ A/B	A/A 5	A/B 15	B/B 9			A/A 26	A/B 24	B/B 4	1.138	0.566	**0.444**
		B	♀ A/B ♂ A/B	A/A 8	A/B 16	B/B 6			A/A 22	A/B 24	B/B 14	0.750	0.687	0.400
	CFGD056	A	♀ A/B ♂ A/null	A/B 4	B/null 4	A/(A or null) 24			A/A 27	A/B 10	B/B 18	8.000	0.018	0.182
	CFFD067	B	♀ A/B ♂ C/null	A/C 8	B/C 8	A/null 6	B/null 8		A/A 28	A/B 8	B/B 20	0.400	0.940	0.143
	CFLD047	A	♀ A/B ♂ C/C	A/C 18	B/C 12				A/A 12	A/B 25	B/B 23	1.200	0.273	0.417
	CFBD159	C	♀ A/B ♂ A/C	A/A 11	A/C 4	A/B 11	B/C 6		A/A 10	A/B 28	B/B 15	4.750	0.191	0.528
LG9	CFLD044	C	♀ A/B ♂ C/D	A/C 10	A/D 6	B/C 4	B/D 11		A/A 25	A/B 12	B/B 15	4.226	0.238	0.231
	CFFD147	A	♀ A/B ♂ A/C	A/A 10	A/C 7	A/B 7	B/C 7		A/A 24	A/B 6	B/B 18	0.871	0.832	0.125
		B	♀ A/B ♂ C/D	A/C 9	A/D 9	B/C 6	B/D 10		A/A 20	A/B 6	B/B 18	1.059	0.787	0.136

Table 1 (continued)

Linkage group	Locus	Family	Parental genotypes	Genotypes of control progeny					Genotypes of gynogenetic progeny			χ^2	*P* value	*y*
		C	♀ A/B ♂ B/B	A/B 16	B/B 17				A/A 21	A/B 15	B/B 21	0.030	0.862	0.263
	CFHP060	A	♀ A/B ♂ A/B	A/A 9	A/B 11	B/B 10			A/A 24	A/B 8	B/B 28	2.200	0.333	0.133
	CFFD061	A	♀ A/B ♂ C/D	A/C 6	A/D 13	B/C 4	B/D 9		A/A 12	A/B 23	B/B 22	5.750	0.124	0.404
		B	♀ A/B ♂ C/null	A/C 7	B/C 9	A/null 6	B/null 8		A/A 10	A/B 22	B/B 20	0.667	0.881	0.423
		C	♀ A/B ♂ A/null	A/B 7	B/null 9	A/(A or null) 16			A/A 6	A/B 30	B/B 16	0.250	0.882	0.577
LG10	CFFD071	A	♀ A/B ♂ A/A	A/A 11	A/B 21				A/A 18	A/B 8	B/B 20	3.125	0.077	0.174
		C	♀ A/B ♂ A/A	A/A 16	A/B 14				A/A 16	A/B 16	B/B 10	0.133	0.715	0.381
	CFJD077	A	♀ A/B ♂ C/D	A/C 7	A/D 9	B/C 6	B/D 9		A/A 8	A/B 30	B/B 20	0.871	0.832	0.517
	CFAD039	A	♀ A/B ♂ A/B	A/A 8	A/B 19	B/B 3			A/A 16	A/B 32	B/B 8	3.800	0.150	0.571
		B	♀ A/B ♂ B/B	A/B 24	B/B 7				A/A 14	A/B 28	B/B 10	8.895	0.003	0.538
		C	♀ A/B ♂ C/D	A/C 8	A/D 5	B/C 8	B/D 11		A/A 25	A/B 16	B/B 11	2.250	0.522	0.308
LG11	CFBD146	A	♀ A/B ♂ C/C	A/C 18	B/C 14				A/A 24	A/B 8	B/B 26	0.500	0.480	0.138
	CFAD099	A	♀ A/B ♂ C/D	A/C 3	A/D 5	B/C 8	B/D 14		A/A 25	A/B 5	B/B 25	9.200	0.027	0.091
		B	♀ A/B ♂ C/D	A/C 8	A/D 9	B/C 8	B/D 7		A/A 15	A/B 15	B/B 24	0.250	0.969	0.278
	CFLD009	B	♀ A/B ♂ A/B	A/A 14	A/B 12	B/B 6			A/A 14	A/B 22	B/B 18	6.000	0.050	0.407
	CFAD213	A	♀ A/B ♂ A/B	A/A 5	A/B 19	B/B 7			A/A 7	A/B 32	B/B 15	1.839	0.399	0.593
		B	♀ A/B ♂ C/C	A/C 19	B/C 17				A/A 12	A/B 28	B/B 6	0.111	0.739	0.609
	CFBD204	A	♀ A/B ♂ B/C	A/B 7	A/C 4	B/B 6	B/C 12		A/A 12	A/B 23	B/B 13	4.793	0.188	0.479
		C	♀ A/B ♂ C/D	A/C 4	A/D 12	B/C 11	B/D 5		A/A 2	A/B 40	B/B 10	6.250	0.100	0.769
LG12	CFKD091	C	♀ A/B ♂ C/D	A/C 12	A/D 5	B/C 6	B/D 8		A/A 13	A/B 15	B/B 23	3.710	0.295	0.294
	CFKD096	A	♀ A/B ♂ C/D	A/C 8	A/D 6	B/C 10	B/D 8		A/A 20	A/B 8	B/B 22	1.000	0.801	0.160
		B	♀ A/B ♂ C/D	A/C 11	A/D 6	B/C 4	B/D 11		A/A 16	A/B 10	B/B 22	4.750	0.191	0.208
	CFKD037	A	♀ A/B ♂ C/null	A/C 4	B/C 5	A/null 8	B/null 11		A/A 19	A/B 26	B/B 9	4.286	0.232	0.481
		B	♀ A/B ♂ C/D	A/C 3	A/D 9	B/C 11	B/D 6		A/A 16	A/B 24	B/B 12	5.069	0.167	0.462
LG13	CFKD022	A	♀ A/B ♂ A/C	A/A 6	A/C 7	A/B 12	B/C 7		A/A 14	A/B 14	B/B 14	2.750	0.432	0.333
		B	♀ A/B ♂ C/B	A/C 6	A/B 6	B/C 8	B/B 11		A/A 14	A/B 15	B/B 25	2.161	0.540	0.278
		C	♀ A/B ♂ C/null	A/C 5	B/C 8	A/null 5	B/null 12		A/A 20	A/B 17	B/B 15	4.400	0.221	0.327

Table 1 (continued)

Linkage group	Locus	Family	Parental genotypes	Genotypes of control progeny					Genotypes of gynogenetic progeny			χ^2	*P* value	*y*
	CFOD062	B	♀ A/B ♂ C/C	A/C 15	B/C 17				A/A 16	A/B 18	B/B 14	0.125	0.724	0.375
	CFBD185	A	♀ A/B ♂ C/null	A/C 10	B/C 5	A/null 11	B/null 3		A/A 16	A/B 34	B/B 8	6.172	0.104	0.586
		B	♀ A/B ♂ B/null	A/B 4	A/null 9	B/(B or null) 17			A/A 24	A/B 18	B/B 9	2.200	0.333	0.353
		C	♀ A/B ♂ C/null	A/C 8	B/C 5	A/null 3	B/null 11		A/A 9	A/B 32	B/B 19	5.444	0.142	0.533
LG14	CFBD023	A	♀ A/B ♂ B/B	A/B 18	B/B 4				A/A 12	A/B 10	B/B 22	8.909	0.003	0.227
	CFBD222	B	♀ A/B ♂ B/C	A/B 16	A/C 2	B/B 5	B/C 8		A/A 24	A/B 21	B/B 12	14.032	0.003	0.368
		C	♀ A/B ♂ A/B	A/A 6	A/B 17	B/B 9			A/A 9	A/B 15	B/B 18	0.688	0.709	0.357
	CFJD047	C	♀ A/B ♂ C/D	A/C 7	A/D 12	B/C 10	B/D 3		A/A 17	A/B 22	B/B 9	5.750	0.124	0.458
	CFMSP003	B	♀ A/B ♂ A/B	A/A 6	A/B 19	B/B 7			A/A 10	A/B 34	B/B 10	1.188	0.552	0.630
LG15	CFGD094	C	♀ A/B ♂ C/D	A/C 12	A/D 3	B/C 10	B/D 7		A/A 16	A/B 16	B/B 18	5.750	0.124	0.320
	CFBD169	B	♀ A/B ♂ C/C	A/C 20	B/C 12				A/A 28	A/B 2	B/B 18	2.000	0.157	0.042
	CFID005	A	♀ A/B ♂ B/C	A/B 11	A/C 4	B/B 5	B/C 10		A/A 24	A/B 20	B/B 16	4.933	0.177	0.333
		B	♀ A/B ♂ C/C	A/C 18	B/C 13				A/A 12	A/B 34	B/B 6	0.806	0.369	0.654
		C	♀ A/B ♂ C/D	A/C 12	A/D 5	B/C 10	B/D 5		A/A 8	A/B 30	B/B 10	4.750	0.191	0.625
LG16	CFMSM014	A	♀ A/B ♂ C/C	A/C 23	B/C 9				A/A 24	A/B 6	B/B 26	6.125	0.013	0.107*
		B	♀ A/B ♂ A/C	A/A 4	A/C 4	A/B 6	B/C 4		A/A 8	A/B 40	B/B 8	0.667	0.881	0.714*
	CFAD074	B	♀ A/B ♂ C/D	A/C 9	A/D 7	B/C 7	B/D 7		A/A 18	A/B 4	B/B 26	0.400	0.940	0.083
		C	♀ A/B ♂ A/C	A/A 9	A/C 12	A/B 5	B/C 5		A/A 20	A/B 10	B/B 12	4.484	0.214	0.238
	CFBD048	A	♀ A/B ♂ C/null	A/C 4	B/C 8	A/null 10	B/null 10		A/A 22	A/B 12	B/B 14	3.000	0.392	0.250
		B	♀ A/B ♂ C/D	A/C 7	A/D 9	B/C 7	B/D 9		A/A 16	A/B 18	B/B 22	0.500	0.912	0.321
		C	♀ A/B ♂ C/null	A/C 8	B/C 10	A/null 2	B/null 10		A/A 22	A/B 22	B/B 8	5.733	0.125	0.423
	CFFD041	A	♀ A/B ♂ C/D	A/C 9	A/D 9	B/C 5	B/D 9		A/A 22	A/B 18	B/B 14	1.500	0.682	0.333
		B	♀ A/B ♂ A/C	A/A 3	A/C 9	A/B 9	B/C 9		A/A 29	A/B 22	B/B 9	3.600	0.308	0.367
		C	♀ A/B ♂ A/C	A/A 11	A/C 12	A/B 3	B/C 5		A/A 12	A/B 24	B/B 10	7.581	0.056	0.522
LG17	CFOD056	A	♀ A/B ♂ A/C	A/A 6	A/C 5	A/B 10	B/C 10		A/A 18	A/B 18	B/B 18	2.677	0.444	0.333
		B	♀ A/B ♂ C/null	A/C 7	B/C 7	A/null 9	B/null 7		A/A 12	A/B 18	B/B 14	0.400	0.940	0.409
		C	♀ A/B ♂ C/D	A/C 7	A/D 4	B/C 10	B/D 9		A/A 28	A/B 15	B/B 17	2.800	0.424	0.250

Table 1 (continued)

Linkage group	Locus	Family	Parental genotypes	Genotypes of control progeny					Genotypes of gynogenetic progeny			χ^2	*P* value	*y*
	CFLD144	A	♀ A/B ♂ C/D	A/C 9	A/D 5	B/C 8	B/D 7		A/A 10	A/B 24	B/B 16	1.207	0.751	0.480
		B	♀ A/B ♂ C/D	A/C 8	A/D 6	B/C 6	B/D 12		A/A 18	A/B 22	B/B 16	3.000	0.392	0.393
		C	♀ A/B ♂ C/null	A/C 3	B/C 15	A/null 7	B/null 2		A/A 15	A/B 27	B/B 18	15.519	0.001	0.450
LG18	CFAD019	A	♀ A/B ♂ C/D	A/C 10	A/D 6	B/C 9	B/D 6		A/A 20	A/B 2	B/B 28	1.645	0.649	0.040
	CFBD224	C	♀ A/B ♂ B/null	A/B 10	A/null 4	B/(B or null) 14			A/A 21	A/B 18	B/B 21	2.571	0.276	0.300
	CFCD126	A	♀ A/B ♂ A/C	A/A 7	A/C 7	A/B 8	B/C 10		A/A 14	A/B 18	B/B 15	0.750	0.861	0.383
		B	♀ A/B ♂ A/C	A/A 6	A/C 7	A/B 9	B/C 10		A/A 10	A/B 14	B/B 20	1.250	0.741	0.318
	CFAD184	C	♀ A/B ♂ C/null	A/C 9	B/C 5	A/null 5	B/null 13		A/A 21	A/B 18	B/B 12	5.500	0.139	0.353
LG19	CFE15	A	♀ A/B ♂ A/A	A/A 14	A/B 17				A/A 12	A/B 23	B/B 12	0.290	0.590	0.489
	CFAD013	A	♀ A/B ♂ C/null	A/C 8	A/null 14	B/C 5	B/null 6		A/A 13	A/B 11	B/B 23	5.909	0.116	0.231
		B	♀ A/B ♂ B/C	A/B 6	A/C 8	B/B 12	B/C 7		A/A 25	A/B 9	B/B 14	2.515	0.473	0.188
	CFLD060	A	♀ A/B ♂ C/D	A/C 9	A/D 9	B/C 8	B/D 2		A/A 12	A/B 36	B/B 9	4.857	0.183	0.632

When determined in two or three families, homogeneity of *y* was tested; *y* values with asterisks were heterogeneous ($P<0.01$) by χ^2 analysis. Bolded *y* values indicate unequal proportions of homozygotes at the 5 % level after Bonferroni correction. Underlined loci were apparently incongruent with previous genetic map information (Zhan et al. 2009)

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