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Influence of epistatic segregation distortion loci on genetic marker linkages in Japanese flounder

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ABSTRACT

For genetic linkage analysis of Japanese flounder, 160 doubled haploids (DH) were artificially produced using mitotic gynogenesis and were genotyped for 458 simple sequence repeat (SSR) markers, 101 of which show distortional segregation. The genetic linkage map was constructed by modifying recombination fractions between the distorted markers. Between the corrected and uncorrected genetic maps, there were considerable differences in genetic distance, but not in relative locations among markers. Using a liability model, a segregation distortion locus (SDL), with an additive genetic effect of 1.772, was mapped between markers BDHYP387 and Poli56TUF of chromosome 24 in the corrected genetic map. Additionally, six pairs of epistatic SDLs were identified on chromosomes 1, 5, 8, 9, 23, and 24. Changes in genetic distances between markers did not occur on chromosome regions with main effect SDLs. However, most chromosome regions where genetic distances changed covered the detected epistatic SDLs. This study concluded that epistatic SDLs decrease linkages between markers and lengthen genetic distances in Japanese flounder. This finding has been partially validated in other DH populations derived from three female Japanese flounders.

1. Introduction

Genetic linkage maps with microsatellite markers have been constructed to identify quantitative trait loci (QTL), marker-assisted selection (MAS), and evolutionary traits in economically important fish species, such as salmon [1,2], tilapia [3], European sea bass [4], rainbow trout [5], sea bream [6], barramundi [7], catfish [8], grass carp [9], Japanese flounder [10], Asian sea bass [11,12], mandarin fish [13], large yellow croaker [14,15], turbot [16] and blunt snout bream [17]. For Japanese flounder, four genetic linkage maps have been published by Coimbra, et al. [18], Jung-Ha Kang [19], Castañosánchez, et al. [10] and Song, et al. [20]. The initial two maps were sparse, only identifying 230 and 463 markers. A “second generation” genetic linkage map has been constructed with 1375 markers, spanning 1147.7 cm and 833.8 cm long, with average intervals of 5.0 cm and 4.4 cm in males and females, respectively. In comparison, a high-density microsatellite genetic linkage map reported by Song, et al. [21] covered a genome length of 1763.3 cm using 1487 markers, so that average interval reached to 1.22 cm. This difference in marker density lied in more unique positions located in the map than the second generation genetic map. However, when constructing genetic linkage maps, some distorted markers were excluded, which not only omitted genetic information but also may show decreased marker density.

Marker segregation distortion, known as non-Mendelian or skewed segregation of markers, has been observed in many aquatic experimental systems [20,22–27]. The distortion was controlled by segregation distortion locus (SDL). As the presumption, at a SDL the survival changes among some classes of gametes before fertilization or viability differences of SDL genotypes post-fertilization but before genotype scoring resulted in the segregation distortion of the marker linked to the SDL [28]. In constructing a genetic linkage map, non-Mendelian segregation of markers can affect the estimation of genetic distance between two markers and the order of markers in the same linkage group [29–31]. Additionally, marker segregation distortion affects the power of detecting QTL dominance [32] and epistatic effects [33], but is not always harmful to QTL mapping for additive effects.

SDLs, namely some chromosomal regions frequently deviate from Mendelian ratios [34,35]. Based on the marker segregation distortion hypothesis, fitness and liability models have been developed to map main effect SDLs. A liability model was able to distinguish systematic environmental effects and detect SDLs with more power than the fitness model [36]. The interaction between SDLs, specifically epistasis, was

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strongly associated with segregation distortion [37,38]. Identification of epistatic SDLs has deciphered genetic mechanisms for inbreeding depression [39], reduced hybrid sterility [40–46] and hybrid non-viability [47,48].

This study produced 160 doubled haploid (DH) Japanese flounder via mitotic gynogenesis, and genotyped 458 simple sequence repeat (SSR) markers. To investigate the influence of epistatic segregation distortion loci on genetic marker linkages, a genetic linkage map containing all genotyped markers, was reconstructed by modifying recombination fractions between distorted markers. Based on the genetic map, epistatic SDLs were located using a liability model with epistatic and main effect SDLs.

2. Material and methods

2.1. DH population

The diploid Japanese flounder (*Paralichthys olivaceus*) population was produced via mitotic gynogenesis that firstly activated eggs with inactivated sperm by ultraviolet rays, and then blocked the first mitotic division by hydrostatic pressure treatment [49]. During gynogenesis, sperm do not genetically contribute to the offspring but stimulate embryogenesis. Red sea bream (*Pagrus major*) sperm was used to initiate development of flounder eggs, which ensured that any surviving offspring only inherited genes from the female Japanese flounder. One female produced 160 healthy DH individuals which were used to analyze the influence of epistatic segregation distortion loci on genetic marker linkages by constructing the genetic linkage map and mapping SDLs.

2.2. Microsatellite marker

A total of 1007 type II SSR markers were collected from the GenBank/EMBL/DDBJ database. In addition, mono-, di-, tri- and tetranucleotide SSRs were screened from 15,268 EST sequences using Tandem repeats finder [50]. Primers were designed using PRIMER3 software [51]. In total, 484 EST-SSR primer pairs were produced and named by starting with "BDHYP." Parental DNA was genotyped to evaluate polymorphisms of these markers.

The PCR reaction mixture (15 μl) contained 30–50 ng genomic DNA, 2 pmol of each primer, 1 U Taq polymerase (Takara), and 1 PCR buffer (50 mM of KCl, 10 mM Tris-HCl, and 1.5 mM $MgCl_2$; pH 8.3). PCR amplification was performed under the following conditions: 1) 3 min at 94 °C for initial denaturation, 2) 25 cycles of 30s at 94 °C, 30s at 55–62 °C for annealing, and 30s at 72 °C, and 3) 10 min at 72 °C. PCR products were electrophoresed on 8% (*w/v*) denatured polyacrylamide gel (19:1 acrylamide: bis-acrylamide and 7 M urea) and identified using silver nitrate staining [52]. Amplification fragments were analyzed using Gel-Pro Analyzer 4.5 software to genotype markers.

2.3. Mapping analyses

A Chi-square test, with Bonferroni adjustment, was used to identify markers with distorted Mendelian segregation. In many studies, when segregation tests were significant, distorted markers were often excluded from the constructed genetic linkage map, which led to the reduced marker density. All genotyped markers were used to construct the linkage map and to modify the effects of epistatic SDLs on linkage among markers. The map was created using DistortedMap software [53]. In the marker analysis, genotyping errors and missing genotypes were corrected and estimated using multipoint methods [53–55].

The following steps outline how the liability model was used to map epistatic SDLs:

In a DH population of n individuals, there were two genotypes at each SDL. Parameters a_k and a_j were additive genetic effects of the kth and jth SDLs, i_{jk} was the epistatic effect between the kth and jth SDLs, and $\Phi(x)$ was the normal distribution function with a cumulative probability.

(1) Calculate the modified conditional probabilities p_k and p_j at the kth and jth SDLs on three or four flanking markers in the DH population [33] to obtain conditional probabilities for four genotype combinations at the two SDLs as $P_{kj} = p_k p_j$ where $k, j = 1, 2$.
(2) Calculate the individual survival probabilities of four genotype combinations $\omega_{kj} = \Phi[(-1)^k a_1 + (-1)^j a_2 + (-1)^k(-1)^j i_{kj}]$ to estimate the expected survival frequencies $f_{kj} = \frac{\omega_{kj}}{\sum_{k=1}^{2}\sum_{j=1}^{2}\omega_{kj}}$ where $k, j = 1, 2$.
(3) Calculate the log-likelihood function $L(a_k, a_j, i_{kj}) = \Sigma_{l=1}^{n} \ln(\Sigma_{k=1}^{2}\Sigma_{j=1}^{2} P_{kj} f_{kj})$ to form the likelihood ratios $LR = -2(L(a_k, a_j, i_{kj}) - L(0, a_j, i_{kj})$ for statistically inferring main effect SDLs, while $LR = -2(L(a_k, a_j, i_{kj}) - L(a_k, a_j, 0)$ for epistatic SDLs.
(4) Repeat steps (1)–(3) through two-dimensional scans of step length of 1 cm.

Because log-likelihoods were nonlinear functions about the estimated parameters, the maximum likelihood estimates of additive and epistatic effects were analyzed by nonlinear regression analysis using the "nlm" function in R software. To identify SDLs using likelihood ratio (LR) tests, empirical critical values were determined by permutation tests [56]. By repeatedly shuffling the relationships among marker genotypes, a series of maximum LRs were calculated, from the distribution of which the critical threshold was obtained. The R code for mapping epistatic SDLs was freely available upon request.

The heritability of SDLs, trait variance contributed by SDLs on the liability scale was determined by

$$h^2_{a\ or\ i} = \frac{a^2\ or\ i^2}{a^2\ or\ i^2 + 1}$$

3. Results

3.1. Marker segregation distortion

A total of 622 polymorphic SSR markers were genotyped for 160 DH individuals, of which 506 were identified as independent markers by deleting the repeated markers among which all individuals had the same genotypes. The 506 independent markers were clustered into 24 linkage groups (LGs) corresponding to the 24 chromosomes of Japanese flounder. Fig. 1 depicted gene frequencies of the markers across the 24 LGs. Chi-square tests for markers showed that 101 markers were non-Mendelian segregation markers at significance level of 9.88e-05 (0.05/506). As displayed in Fig. 1, the distorted markers were found on more than half of LGs involving chromosomes 1, 3, 7, 9, 11, 20, and 24. The markers drastically skewed the Mendelian ratio of 1:1, mainly on chromosomes 1, 3, 7, and 24. Specifically, the population contained almost only one kind of genotype at the end of chromosome 24.

3.2. Construction of genetic linkage map

Without modification of segregation distortion, 574 of 622 polymorphic SSR markers were mapped at 458 unique positions on 24 LGs, and 48 markers were not linked to any LGs. Therefore, 458 genetic markers were used to construct the genetic linkage map [57]. The linkage map spanned 1214.7 cm, and the average interval between adjacent unique marker loci was 2.65 cm long.

After modifying marker segregation distortion, the genetic linkage map was reconstructed with DistortedMap software (Fig. 2). Overall, the corrected genetic map was different considerably from the uncorrected map in genetic distance among nearly half of all markers, but was same in order of all markers. The differences in genetic distance occurred on all chromosomes where the distorted markers resided,