

A high-density genetic linkage map for Chinese perch (*Siniperca chuatsi*) using genotyping-by-sequencing (GBS)

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Abstract

Background: Chinese perch, *Siniperca chuatsi* (Basilewsky), is one of the most commercially important cultured fishes in China. In the present study, a high-density genetic linkage map of Chinese perch was constructed by genotyping-by-sequencing (GBS) technique with an F1 mapping panel containing 190 progenies. **Result:** A total of 2328 SNPs were assigned to 24 linkage groups (LGs), agreeing with the chromosome haploid number in this species ($n = 24$). The sex-averaged map covered 97.9% of the Chinese perch genome, with the length of 1694.3 cM and a marker density of 0.7 cM/locus. The number of markers per LG ranged from 57 to 222, with a mean of 97. The length of LGs varied from 43.2 cM to 108.2 cM, with a mean size of 70.6 cM. The recombination rate of females was 1.50 : 1, which was higher than that of males. In order to better understand the distribution pattern of segregation distortion between the two sexes of Chinese perch, the skewed markers were retained and used to reconstruct the sex-specific maps. The 16 SDRs were identified on 10 LGs of the female map, while 12 SDRs on 8 LGs of the male map. Among these LGs, 6 LGs matched between the sex-specific maps. **Conclusion:** This high-density linkage map could provide a solid basis for identifying QTLs associated with economically important traits, and for implementing marker-assisted selection breeding of Chinese perch.

Background

Chinese perch, *Siniperca chuatsi* (Basilewsky), is one of the most commercially important cultured fishes in China [1]. It has been massively cultured throughout the country, especially in Guangdong Province due to its excellent taste, fast growth, and broad temperature tolerance range [2]. Its production will grow rapidly in the coming years, with the improvement of people's living standards and the exploration of overseas market. However, in the past three decades, the artificial breeding projects of Chinese perch only focused on the quantity of fish fry, but not on its quality. The genetic diversity of *S. chuatsi* stocks has been dramatically decreased during several generations, leading to the recession of excellent properties, such as growth performance and disease resistance. Therefore, genetic improvement programmes should be performed in *S. chuatsi* to consolidate and improve its excellent properties. Generally, the important agronomical traits are governed by interactions of multiple genetic loci which are difficult to be identified by traditional quantitative genetics methods [3].

The molecular markers have been an effective tool to detect and genetically locate the quantitative trait loci (QTLs) for genetic improvement [4]. Marker-assisted selection (MAS), utilizing molecular markers as indicators for indirect selection of a targeted trait, exhibits considerable advantages over conventional breeding approaches, especially for those traits which are difficult to measure and record on a continuous quantitative scale, and for those species with long generation times [5, 6]. Genetic linkage map construction using polymorphic markers is one of the most crucial steps for MAS projects, and also provides a framework for comparative mapping study and chromosome-level assembly of whole-genome sequences [7–9].

The advances in technology of molecular marker exploitation and linkage analysis made it possible to construct genetic linkage map for diverse species [10]. Due to its high abundance, uniform distribution, biallelic nature and genetic stability, single nucleotide polymorphisms (SNPs) has gradually replaced traditional molecular markers such as amplified fragment length polymorphism (AFLP) and microsatellites (SSRs) in molecular genetics studies [11–14]. Genotyping-by-sequencing (GBS) could discover genome-wide SNPs in a

rapid and cost-effective manner, which allows the processes of sequencing, discovery, and scoring of massive SNPs simultaneously in a single procedure [15]. GBS has successfully been applied to construct high-density linkage maps in a variety of different species [16–18].

The genomics studies and genetic analysis of the genetic improvement and artificial breeding of *S. chuatsi* are still in infancy. Although molecular markers have been reported previously [19–21], these studies could not meet the increasing industry demands, yet. In the present study, we used a panel of SNPs developed by GBS technique to produce a high-density genetic linkage map of *S. chuatsi*. The map developed in this study could be an important tool in elucidating genome structure and organization, anchoring the scaffolds in ongoing genome sequencing project, identifying QTLs associated with economically important traits, and implementing MAS for genetic improvement in Chinese perch breeding.

Results

SNP Discovery in *S. chuatsi* Using GBS approach

To identify SNP markers present in the mapping family for map construction, ApeKI reduced representation libraries were constructed and sequenced using the Illumina HiSeq4000. After filtering the dirty raw reads, a total of 974.48 M reads were obtained with a mean of approximately 10.55 and 5.02 million reads for parents and offspring, respectively. A total of 65,535 putative GBS markers were discovered, and of which, 12,466 were informative. From these 12,466 informative markers, 2519 high-quality SNPs were retained for linkage analyses.

Linkage mapping

Linkage analyses were conducted by Joinmap 4.0 software with LOD threshold of 5.0 for female and male maps, and of 6.0 for sex-averaged map. After eliminating the markers with significant segregation distortion, there were 24 linkage groups in the final linkage maps with 1030, 880 and 2328 SNPs markers in the male, female and sex-averaged map, respectively (Table 1; Table 2; Figure 1). The male, female and sex-averaged map covered a total genetic distance of 1885.9 cM, 1262.8 cM, and 1694.3 cM respectively, with an average interval of 1.2 cM, 2.1 cM, and 0.7 cM (Table 2).

Marker distribution and intermarker space

The SNP markers located in the linkage groups were unevenly distributed. LG 1 was the largest linkage group in all the three maps, containing a total of 113 (male map), 169 (female map), and 222 markers (sex-averaged map), respectively. LG 24 was the smallest one, with 7, 13 and 37 markers, respectively (Table 1). To further investigate the marker distribution, intermarker space of each linkage group was calculated in sex-averaged map. Most intervals in this sex-averaged map were less than 5 cM with its percentage of 97.9% (2256/2304) (Figure 2).

Estimation of genome size and coverage

The genome sizes of the male, female, and sex-average maps were estimated to be 1328.3 cM, 2010.2 cM, and 1731.0 cM, respectively. Based on these estimated sizes, genome coverages were calculated to be 97.9% (male map), 93.8% (female map) and 95.1% (sex-average map), respectively. The haploid genome of *S. chuatsi* was reported to be approximately 0.8 Gb in a previous study [22]. In our study, linkage map length was estimated to be 1731.0 cM. Thus the relationship of physical to genetic distance could be estimated to be 462.2 Kb/cM. Therefore, the estimated physical distance between the adjacent markers ranged from 0 Kb to 9.2 Mb with an average of 323.5 Kb in the sex-averaged map.

Segregation distortion

In the present study, 150 and 239 segregating markers ($P < 0.05$) were assembled into the SD male and female map, respectively (Table 3). These markers accounted for 12.7% in the SD male map and 21.4% in the SD female map. The segregation distortion markers among LGs also showed an uneven distribution. In the male map, the percentage of segregation distortion markers ranged from 0.0% (MSD-LG12, MSD-LG15, MSD-LG23, MSD-LG24) to 41.7% (MSD-LG22), while in the female map, it ranged from 0.0% (FSD-LG5, FSD-LG6) to 90.8% (FSD-LG24). The 12 and 16 SDRs were detected on 8 LGs in the male map and 10 LGs in the female map, respectively. Among these linkage groups, 6 LGs containing SDRs were shared by male map and female map.

Sex differences in recombination rate

The differences in both the number of markers and recombination rate were observed between the sex-specific genetic linkage maps. Overall, the marker number in the male map was larger than that in the female map, whereas, the total length was shorter. The relative recombination ratio between female and male was 1.5/1 for the overall LGs, but the recombination ratios were different for individual LG, ranging from 0.5 to 10.6 (Table 4). On 18 out of 24 LGs, higher recombination rates were observed in male map than in female map. While, on the other 6 LGs, recombination rates were higher in female map than in male map.

Discussion

In this study, the linkage map was described for *S. chuatsi*, a commercially important freshwater fish in China, based on 2328 SNP markers. These markers were located on 24 LGs, consistent with the haploid chromosome number of *S. chuatsi* ($n = 24$). The sex-averaged total map length was 1694.3 cM, covering 97.9% of the expected length (1731.0 cM). According to the *S. chuatsi* genome size of 800 Mb reported in previous study and the mean intermarker space of 0.7 cM estimated in our study, the present map contained a marker approximately 0.3235 Mb, could provide sufficient marker density for QTL mapping and other genetic or genomic analyses. The majority of the marker intervals were found to be less than 5 cM (97.9%), and only 0.7% were more than 10 cM in the present genetic linkage map of *S. chuatsi*, indicating that SNP markers were well-distributed along the whole chromosomes of *S. chuatsi* (Figure 2). Large intervals observed in genetic linkage maps were a common phenomenon, which has been reported in other animals [23–26]. These large intervals indicated that some regions in the genome exhibited the very little recombination or they were highly conserved regions. In these regions, the informative SNP markers were reported to be rare or difficult to identify [27, 28].

Sex difference in recombination rates was a common phenomenon across species with the least recombination usually observed in the heterogametic sex [29]. Though no heteromorphic chromosomes were reported in fishes [30], similar sex difference in recombination rates was reported in fish, such as a ratios of 1.18:1 in the white grouper [31], 2.00:1 in grass carp [10], 1.45:1 in the kelp grouper [32], 1.03:1 in orange-spotted groupers [33], and 1.69:1 in Arctic char [34]. This study found that females also had a higher recombination rate (1.50:1) than male for *S. chuatsi*.

The knowledge of relative recombination rates of females and males is of great practical significance. High recombination rate plays a positive role in distinguishing closely-linked markers, which could improve the linkage map resolution for QTL mapping of economic traits. In contrast, low recombination rate is helpful for identifying the approximate location of mutation in a chromosome arm [30]. Moreover, double mutants in *cis* with a low recombination rate were reported to be linked frequently, which was extremely useful for epistasis analysis [30]. Therefore, future genetic research such as QTL mapping could take the recombination differences between sexes into consideration in *S. chuatsi* and other *Siniperca* species.

Segregation distortion is a phenomenon that genotype frequencies at a locus deviate from their Mendelian expectations [35], and it is considered to be a feature of genetic maps in many species, such as red drum [6], Atlantic Killifish [36], bighead carp [37], and Nile tilapia [38]. Many studies have already reported that inclusion of distorted markers in map construction has slight impact on the accuracy for determining marker orders and map length [39, 40]. For better understanding the distribution pattern of segregation distortion between the two sexes in *S. chuatsi*, all the distorted markers were retained and used to reconstruct the sex-specific maps. Finally, 150 and 239 distorted markers were integrated into the male and female map, respectively.

In the present study, part of segregation-distorted loci was randomly scattered on the sex-specific maps of *S. chuatsi*, which could be caused by abiological factors, such as the bias generated from the GBS by which sequencing is difficult in highly repetitive regions and data points missing in SNP calling. However, majority of distorted SNPs were clustered on some LGs and/or in some regions within an individual LG. Such a clustering is impossible to result from abiological factors, Instead, it might be due to SDR, a region containing at least three adjoining markers exhibiting distorted segregation. It was reported that SDRs might be related to deleterious genes that potentially affected survival and fitness of organism [41, 42]. In the present study, 16 SDRs were identified on 10 LGs in the female map, while 12 SDRs were found on 8 LGs in the male map. Among these LGs, 6 LGs showed corresponding relations between the male and female maps. Therefore, SDRs on the 6 shared LGs might be used to identify potential lethal or deleterious genes on certain chromosomes of *S. chuatsi*, thus providing useful instruction for breeding projects.

Conclusions

In the present study, a high-density genetic linkage map was constructed for Chinese perch using genotyping-by-sequencing (GBS) technique with an F1 mapping panel containing 190 progenies. A total of 2328 SNPs were assigned to 24 linkage groups (LGs), agreeing with the chromosome number of haploid genome in this species ($n = 24$). The sex-averaged map spanned 1694.3 cM covering 97.9% of the Chinese perch genome, with one SNP marker in every 0.7 cM. The 16 SDRs were identified on 10 LGs in the female map, while 12 SDRs on 8 LGs in the male map. The SDRs on the shared 6 LGs by both female map and male map might be used to identify

potential lethal or deleterious genes on certain chromosomes of *S. chuatsi*, thus further providing useful instruction for breeding projects.

Methods

Mapping family and DNA isolation

A F1 full-sib family for linkage map construction was generated by mating a female with a male via artificial insemination at the Yangchun Fish Farm (Yangjiang City, Guangdong Province, P. R. China). Fin clips of parental fish and 190 of 1-month-old fries randomly chosen as the mapping family were sampled and fixed in 100% ethanol for further DNA isolation. Genomic DNA was extracted according to a traditional phenol–chloroform protocol [43]. The genomic integrity was checked by 1.5% agarose gel electrophoresis and the DNA concentrations were assessed using a spectrophotometer (NanoDrop 5000, Thermo Scientific, Wilmington, DE, USA).

Genotyping-by-sequencing

The GBS library was prepared according to the protocol described by Elshire, Glaubitz (15) with slight modification mentioned below. In brief, the genomic DNA of the parents and their 190 F1 offspring was initially digested individually with the ApeKI restriction enzyme (NEB) (recognition cut site 5'-GCWGC-3'), then the digested DNA was ligated with individual-specific barcodes ranging from 4 to 8 nucleotides. Ninety-six ligation products of different samples were pooled and the obtained 2 pools with an approximate size range of 180–480 bp were respectively sequenced on a single lane of the Illumina HiSeq4000 Platform (Illumina Inc., San Diego, CA) by using 100 bp paired-end reads at BGI (Shenzhen, China). Raw sequence datasets obtained in our study were available in the Sequence Read Archive (SRA) database with the accession number: SRP118791.

No reference genome of *S. chuatsi* was available to align raw reads for SNP detection. Therefore, the program Stacks was utilized to discover SNP markers. This program is available for free download from <http://creskolab.uoregon.edu/stacks/> [44]. After the removal of adaptor sequences, short DNA sequences (reads) were sorted to individuals from the sequencing pools by barcode sequences by using the program process_radtags. Reads of each individual were merged into populations and the consensus sequences were generated using the “ustacks” and “cstacks” program. SNP calling and genotyping were conducted by sstacks and genotypes programs, respectively. Any SNP with > 20% missing data was excluded from further segregation analysis.

Map construction

Mendelian segregation at each locus was subjected to Chi-square test. Only SNPs with $P \geq 0.05$ were selected for the map construction. Linkage groups were created with JoinMap 4.0 program under CP algorithm. Four marker segregation types were used in the genetic linkage mapping: Type I (Im × II) was heterozygous in the male (Im) and homozygous in female (II) which was recorded for paternal linkage mapping; Type II (nn × np) were homozygous in the male (nn) and heterozygous in female (np), which was recorded for maternal linkage

mapping; Type III (eg × ef) was heterozygous in both parents with three alleles (two sex-specific alleles and one shared allele); Type IV (hk × hk) was heterozygous in both parents with two common alleles, which was recorded as anchor markers for the final sex-average linkage map. The map distances were calculated by the Kosambi's function [45] with a minimum logarithmic of odds (LOD) threshold of 6.0 for sex-average linkage map and 5.0 for sex-specific linkage maps. The final maps were visualized by using by the Mapchart software version 2.2 [46].

To discuss the distribution of distortion segregation markers between male map and female map, both non-segregation and segregation distortion markers were integrated together for linkage analysis. Segregation distortion region (SDR) was defined as a region containing at least three adjacent distorted loci ($P < 0.05$) [47].

Genome size and coverage estimation

Two different approaches were applied to evaluate the genome length (G_e) of *S. chuatsi*. First, genome estimation size 1 (G_{e1}) was calculated to account for terminal chromosome regions by adding $2 \times D_{av}$ (the average inter-marker distance of the linkage map) to the length of each linkage group [48]. Genome estimation size 2 (G_{e2}) was estimated by multiplying the total length of each linkage group by the factor $(m + 1)/(m - 1)$, where m was the number of markers on each linkage group [49]. The estimated genome size for *S. chuatsi* was taken as the average of the two estimates (G_1 and G_2). Genome coverage was determined as the ratio of the cumulative linkage group length to the estimated genome size.

Abbreviations

Not applicable.

Declarations

Ethics approval and consent to participate

No endangered or protected species were involved in all field studies.. The animal protocol was approved by the Ethical Committee of Huazhong Agricultural University.

Consent for publication

Not applicable.

Availability of data and materials

We declared that materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes, without breaching participant confidentiality.

Competing Interests

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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Author Contributions

WG, SH, CT, YD, LL contributed to data curation. XL gave technical advice and contributed to the study design. WG and SH performed the visualization and wrote the paper. All authors read and approved the final manuscript.

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References

1. Tian CX, Yang M, Lv LY, Yuan YC, Liang XF, Guo WJ, et al. Single Nucleotide Polymorphisms in Growth Hormone Gene and Their Association with Growth Traits in *Siniperca chuatsi* (Basilewsky). International Journal of Molecular Sciences. 2014;15(4):7029–36. doi: 10.3390/ijms15047029. PubMed PMID: WOS:000336841200120.
2. Yi T, Guo W, Liang X, Yang M, Lv L, Tian C, et al. Microsatellite analysis of genetic diversity and genetic structure in five consecutive breeding generations of mandarin fish *Siniperca chuatsi* (Basilewsky). Genetics and Molecular Research. 2015;14(1):2600–7.
3. Ashikari M, Matsuoka M. Identification, isolation and pyramiding of quantitative trait loci for rice breeding. Trends in plant science. 2006;11(7):344–50.
4. Liu R, Wang B, Guo W, Qin Y, Wang L, Zhang Y, et al. Quantitative trait loci mapping for yield and its components by using two immortalized populations of a heterotic hybrid in *Gossypium hirsutum* L. Molecular breeding. 2012;29(2):297–311.

5. Hulata G. Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. *Genetica*. 2001;111(1–3):155–73.
6. Hollenbeck CM, Portnoy DS, Gold JR. A genetic linkage map of red drum (*Sciaenops ocellatus*) and comparison of chromosomal synteny with four other fish species. *Aquaculture*. 2015;435:265–74.
7. Li Y, Liu S, Qin Z, Waldbieser G, Wang R, Sun L, et al. Construction of a high-density, high-resolution genetic map and its integration with BAC-based physical map in channel catfish. *DNA Research*. 2014;22(1):39–52.
8. Aoki J-y, Kai W, Kawabata Y, Ozaki A, Yoshida K, Koyama T, et al. Second generation physical and linkage maps of yellowtail (*Seriola quinqueradiata*) and comparison of synteny with four model fish. *BMC genomics*. 2015;16(1):406.
9. Shao C, Niu Y, Rastas P, Liu Y, Xie Z, Li H, et al. Genome-wide SNP identification for the construction of a high-resolution genetic map of Japanese flounder (*Paralichthys olivaceus*): applications to QTL mapping of *Vibrio anguillarum* disease resistance and comparative genomic analysis. *DNA Research*. 2015;22(2):161–70.
10. Xia JH, Liu F, Zhu ZY, Fu J, Feng J, Li J, et al. A consensus linkage map of the grass carp (*Ctenopharyngodon idella*) based on microsatellites and SNPs. *BMC genomics*. 2010;11(1):135.
11. Nielsen R. Estimation of population parameters and recombination rates from single nucleotide polymorphisms. *Genetics*. 2000;154(2):931–42.
12. Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, et al. Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS one*. 2008;3(10):e3376.
13. Coates BS, Sumerford DV, Miller NJ, Kim KS, Sappington TW, Siegfried BD, et al. Comparative performance of single nucleotide polymorphism and microsatellite markers for population genetic analysis. *Journal of Heredity*. 2009;100(5):556–64.
14. Pootakham W, Ruang-Areerate P, Jomchai N, Sonthirod C, Sangsrakru D, Yoocha T, et al. Construction of a high-density integrated genetic linkage map of rubber tree (*Hevea brasiliensis*) using genotyping-by-sequencing (GBS). *Frontiers in plant science*. 2015;6.
15. Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, et al. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS one*. 2011;6(5):e19379.
16. Ward JA, Bhangoo J, Fernández-Fernández F, Moore P, Swanson J, Viola R, et al. Saturated linkage map construction in *Rubus idaeus* using genotyping by sequencing and genome-independent imputation. *BMC genomics*. 2013;14(1):2.
17. Everett MV, Seeb JE. Detection and mapping of QTL for temperature tolerance and body size in Chinook salmon (*Oncorhynchus tshawytscha*) using genotyping by sequencing. *Evolutionary applications*. 2014;7(4):480–92.
18. İpek A, Yılmaz K, Sıkıcı P, Tangu NA, Öz AT, Bayraktar M, et al. SNP discovery by GBS in olive and the construction of a high-density genetic linkage map. *Biochemical genetics*. 2016;54(3):313–25.

19. Kuang G, Lu S, Zheng S, Wu Q. Isolation and evaluation of 18 microsatellite markers in *Siniperca chuatsi* (Basilewsky). *Molecular ecology resources*. 2009;9(6):1473–5.
20. Liu X, Luo W, Zeng C, Wang W, Gao Z. Isolation of new 40 microsatellite markers in mandarin fish (*Siniperca chuatsi*). *International journal of molecular sciences*. 2011;12(7):4180–9.
21. Huang W, Liang X, Qu C, Cao J, Zhao C, Cao L. Development and characterization of novel polymorphic microsatellite loci in *Siniperca scherzeri* Steindachner and *Siniperca chuatsi* (Basilewsky). *Molecular biology reports*. 2013;40(2):751–6.
22. Cui J, Ren X, Yu Q. Nuclear DNA content variation in fishes. *Cytologia*. 1991;56(3):425–9.
23. Zhang Z-S, Hu M-C, Zhang J, Liu D-J, Zheng J, Zhang K, et al. Construction of a comprehensive PCR-based marker linkage map and QTL mapping for fiber quality traits in upland cotton (*Gossypium hirsutum* L.). *Molecular Breeding*. 2009;24(1):49–61.
24. Bradley KM, Breyer JP, Melville DB, Broman KW, Knapik EW, Smith JR. An SNP-based linkage map for zebrafish reveals sex determination loci. *G3: Genes, Genomes, Genetics*. 2011;1(1):3–9.
25. Huang BE, George AW, Forrest KL, Kilian A, Hayden MJ, Morell MK, et al. A multiparent advanced generation inter-cross population for genetic analysis in wheat. *Plant biotechnology journal*. 2012;10(7):826–39.
26. Shen X, Kwan H, Thevasagayam N, Prakki S, Kuznetsova I, Ngoh S, et al. The first transcriptome and genetic linkage map for Asian arowana. *Molecular ecology resources*. 2014;14(3):622–35.
27. Gan S, Shi J, Li M, Wu K, Wu J, Bai J. Moderate-density molecular maps of *Eucalyptus urophylla* ST Blake and *E. tereticornis* Smith genomes based on RAPD markers. *Genetica*. 2003;118(1):59–67.
28. Zhang N, Zhang L, Tao Y, Guo L, Sun J, Li X, et al. Construction of a high density SNP linkage map of kelp (*Saccharina japonica*) by sequencing Taq I site associated DNA and mapping of a sex determining locus. *BMC genomics*. 2015;16(1):189.
29. Tsigenopoulos CS, Louro B, Chatziplis D, Lagnel J, Vogiatzi E, Loukovitis D, et al. Second generation genetic linkage map for the gilthead sea bream *Sparus aurata* L. *Marine genomics*. 2014;18:77–82.
30. Singer A, Perlman H, Yan Y, Walker C, Corley-Smith G, Brandhorst B, et al. Sex-specific recombination rates in zebrafish (*Danio rerio*). *Genetics*. 2002;160(2):649–57.
31. Dor L, Shirak A, Gorshkov S, Band MR, Korol A, Ronin Y, et al. Construction of a microsatellites-based linkage map for the white grouper (*Epinephelus aeneus*). *G3: Genes, Genomes, Genetics*. 2014;4(8):1455–64.
32. Liu Q, Sakamoto T, Kubota S, Okamoto N, Yamashita H, Takagi M, et al. A genetic linkage map of kelp grouper (*Epinephelus bruneus*) based on microsatellite markers. *Aquaculture*. 2013;414:63–81.
33. You X, Shu L, Li S, Chen J, Luo J, Lu J, et al. Construction of high-density genetic linkage maps for orange-spotted grouper *Epinephelus coioides* using multiplexed shotgun genotyping. *BMC genetics*. 2013;14(1):113.

34. Woram R, McGowan C, Stout J, Gharbi K, Ferguson M, Hoyheim B, et al. A genetic linkage map for Arctic char (*Salvelinus alpinus*): evidence for higher recombination rates and segregation distortion in hybrid versus pure strain mapping parents. *Genome*. 2004;47(2):304–15.
35. Zhan H, Xu S. Generalized linear mixed model for segregation distortion analysis. *BMC genetics*. 2011;12(1):97.
36. Waits ER, Martinson J, Rinner B, Morris S, Proestou D, Champlin D, et al. Genetic linkage map and comparative genome analysis for the Atlantic killifish (*Fundulus heteroclitus*). *Open Journal of Genetics*. 2016;6(01):28.
37. Zhu C, Tong J, Yu X, Guo W, Wang X, Liu H, et al. A second-generation genetic linkage map for bighead carp (*Aristichthys nobilis*) based on microsatellite markers. *Animal genetics*. 2014;45(5):699–708.
38. Zhu H, Liu Z, Lu M, Gao F, Ke X, Huang Z. Screening and identification of microsatellite markers associated with cold tolerance in Nile tilapia *Oreochromis niloticus*. *Genetics and Molecular Research*. 2015;14(3):10308–14.
39. Hackett C, Broadfoot L. Effects of genotyping errors, missing values and segregation distortion in molecular marker data on the construction of linkage maps. *Heredity*. 2003;90(1):33–8.
40. Ma J-Q, Yao M-Z, Ma C-L, Wang X-C, Jin J-Q, Wang X-M, et al. Construction of a SSR-based genetic map and identification of QTLs for catechins content in tea plant (*Camellia sinensis*). *PloS one*. 2014;9(3):e93131.
41. Young WP, Wheeler PA, Coryell VH, Keim P, Thorgaard GH. A detailed linkage map of rainbow trout produced using doubled haploids. *Genetics*. 1998;148(2):839–50.
42. Li L, Guo X. AFLP-based genetic linkage maps of the Pacific oyster *Crassostrea gigas* Thunberg. *Marine Biotechnology*. 2004;6(1):26–36.
43. Taggart J, Hynes R, Prodöuhl P, Ferguson A. A simplified protocol for routine total DNA isolation from salmonid fishes. *Journal of fish biology*. 1992;40(6):963–5.
44. Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH. Stacks: building and genotyping loci de novo from short-read sequences. *G3: Genes, genomes, genetics*. 2011;1(3):171–82.
45. Kosambi D. The estimation of map distances from recombination values. *Annals of Human Genetics*. 1943;12(1):172–5.
46. Voorrips R. MapChart: software for the graphical presentation of linkage maps and QTLs. *Journal of heredity*. 2002;93(1):77–8.
47. Guo W, Tong J, Yu X, Zhu C, Feng X, Fu B, et al. A second generation genetic linkage map for silver carp (*Hypophthalmichthys molitrix*) using microsatellite markers. *Aquaculture*. 2013;412:97–106.
48. Fishman L, Kelly AJ, Morgan E, Willis JH. A genetic map in the *Mimulus guttatus* species complex reveals transmission ratio distortion due to heterospecific interactions. *Genetics*. 2001;159(4):1701–16.

49.Chakravarti A, Lasher LK, Reefer JE. A maximum likelihood method for estimating genome length using genetic linkage data. *Genetics*. 1991;128(1):175–82.

Tables

Table 1 Characteristics of sex-specific and sex-averaged linkage groups of *S. chuatsi*.

Male				Sex-averaged map				Female			
LG	No. of markers	cM	cM/marker	LG	No. of markers	cM	cM/marker	LG	No. of markers	cM	cM/marker
1	169	12.7	0.1	1	222	67.3	0.3	24	7	60.5	8.6
2	60	66.6	1.1	2	201	59.7	0.3	1	113	54.2	0.5
6	51	61.6	1.2	3	121	79.3	0.7	5	51	89.4	1.8
9	42	64.2	1.5	4	110	108.2	1.0	6	51	106.7	2.1
10	41	60.8	1.5	5	108	80.1	0.7	4	53	116.5	2.2
13	36	52.2	1.4	6	105	69.2	0.7	7	48	77.7	1.6
16	32	52.3	1.6	7	104	88.8	0.9	2	60	94.7	1.6
3	57	50.1	0.9	8	99	76.0	0.8	12	33	89.6	2.7
4	54	66.5	1.2	9	98	71.9	0.7	16	28	91.6	3.3
21	25	31.6	1.3	10	98	43.2	0.4	3	57	117.8	2.1
17	32	47.1	1.5	11	97	70.6	0.7	9	46	107.0	2.3
11	38	64.9	1.7	12	91	72.6	0.8	13	33	84.9	2.6
15	35	66.3	1.9	13	88	56.1	0.6	19	16	44.6	2.8
20	28	56.0	2.0	14	86	92.2	1.1	11	37	103.7	2.8
18	29	54.3	1.9	15	83	53.9	0.6	14	31	59.8	1.9
19	29	42.4	1.5	16	80	69.2	0.9	10	43	80.2	1.9
7	49	66.3	1.4	17	79	59.3	0.8	17	24	73.4	3.1
24	13	37.7	2.9	18	78	66.5	0.9	8	47	89.6	1.9
8	43	81.0	1.9	19	78	69.8	0.9	21	15	43.4	2.9
5	54	64.2	1.2	20	76	76.2	1.0	20	15	48.4	3.2
14	36	32.0	0.9	21	66	74.9	1.1	22	12	83.5	7.0
12	38	60.9	1.6	22	66	61.8	0.9	18	21	43.7	2.1
23	18	6.5	0.4	23	57	60.4	1.1	15	30	69.7	2.3
22	21	64.7	3.1	24	37	67.0	1.8	23	9	55.5	6.2

Table 2 Summary of genetic linkage maps of *S. chuatsi*.

Item	Male map	Sex-averaged map	Female map
Linkage groups (LGs)	24	24	24
Total number of markers	1030	2328	880
verage number of markers per LG	42.9	97.0	36.7
Average marker spacing (cM)	1.2	0.7	2.1
Maximum marker spacing (cM)	26.2	19.8	33.7
Average length of per LG (cM)	52.6	70.6	78.6
Map length (cM)	1262.8	1694.3	1885.9
Estimated map length (cM)	1328.3	1731.0	2010.2
Coverage (%)	95.1	97.9	93.8

Table 3 Characteristics of SDL and SDR in sex-specific maps containing SDL of *S. chuatsi*.

ex-averaged map LG	Male map containing SDL			Female map containing SDL		
	LG	No. of SDL	No. of SDRs	LG	No. of SDL	No. of SDRs
1	1	10	1	24	69	2
2	2	3	0	1	8	0
3	6	1	0	5	0	0
4	9	23	2	6	0	0
5	10	9	2	4	7	1
6	13	19	3	7	8	1
7	16	3	0	2	1	0
8	3	1	0	12	9	1
9	4	9	0	16	3	0
10	21	7	1	3	2	0
11	17	3	0	9	2	0
12	11	4	0	13	2	0
13	15	0	0	19	5	0
14	20	5	1	11	10	1
15	18	4	0	14	1	0
16	19	1	0	10	2	0
17	7	8	0	17	31	3
18	24	0	0	8	3	0
19	8	2	0	21	4	0
20	5	15	0	20	7	1
21	14	8	1	22	37	3
22	12	0	0	18	9	1
23	23	0	0	15	1	0
24	22	15	1	23	18	2
Total		150	12		239	16

Table 4 Comparative recombination rates between male and female *S. chuatsi*.

LG	Sex-specific linkage groups	Female: male ratio
1	F-LG24 & M-LG1	4.8
2	F-LG1 & M-LG2	0.8
3	F-LG5 & M-LG6	1.5
4	F-LG6 & M-LG9	1.7
5	F-LG4 & M-LG10	1.9
6	F-LG7 & M-LG13	1.5
7	F-LG2 & M-LG16	1.8
8	F-LG12 & M-LG3	1.8
9	F-LG16 & M-LG4	1.4
10	F-LG3 & M-LG21	3.7
11	F-LG9 & M-LG17	2.3
12	F-LG13 & M-LG11	1.3
13	F-LG19 & M-LG15	0.7
14	F-LG11 & M-LG20	1.9
15	F-LG14 & M-LG18	1.1
16	F-LG10 & M-LG19	1.9
17	F-LG17 & M-LG7	1.1
18	F-LG8 & M-LG24	2.4
19	F-LG21 & M-LG8	0.5
20	F-LG20 & M-LG5	0.8
21	F-LG22 & M-LG14	2.6
22	F-LG18 & M-LG12	0.7
23	F-LG15 & M-LG23	10.6
24	F-LG23 & M-LG22	0.9
Total		1.5

Figures

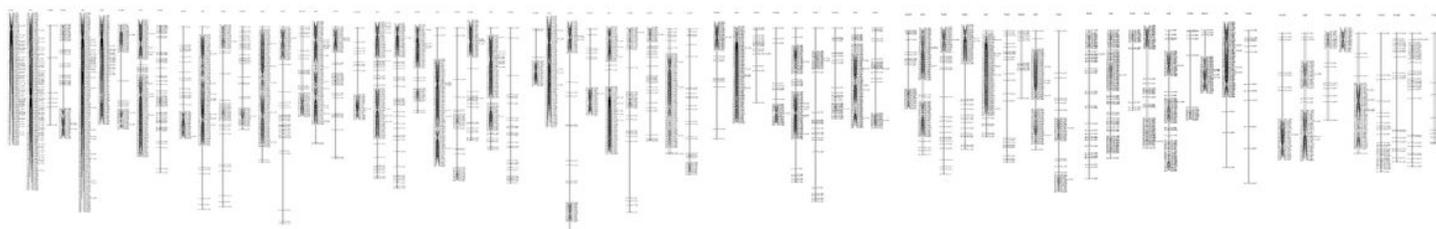


Figure 1

Sex-specific and sex-averaged maps of *S. chuatsi* based on SNPs. The male linkage group (left) is named “M-LG1-M-LG24”; the female linkage group (right) is named “F-LG1-F-LG24”; the sex-averaged linkage group (middle) is named “LG1- LG24”. Total lengths of linkage groups are expressed as Kosambi cM.

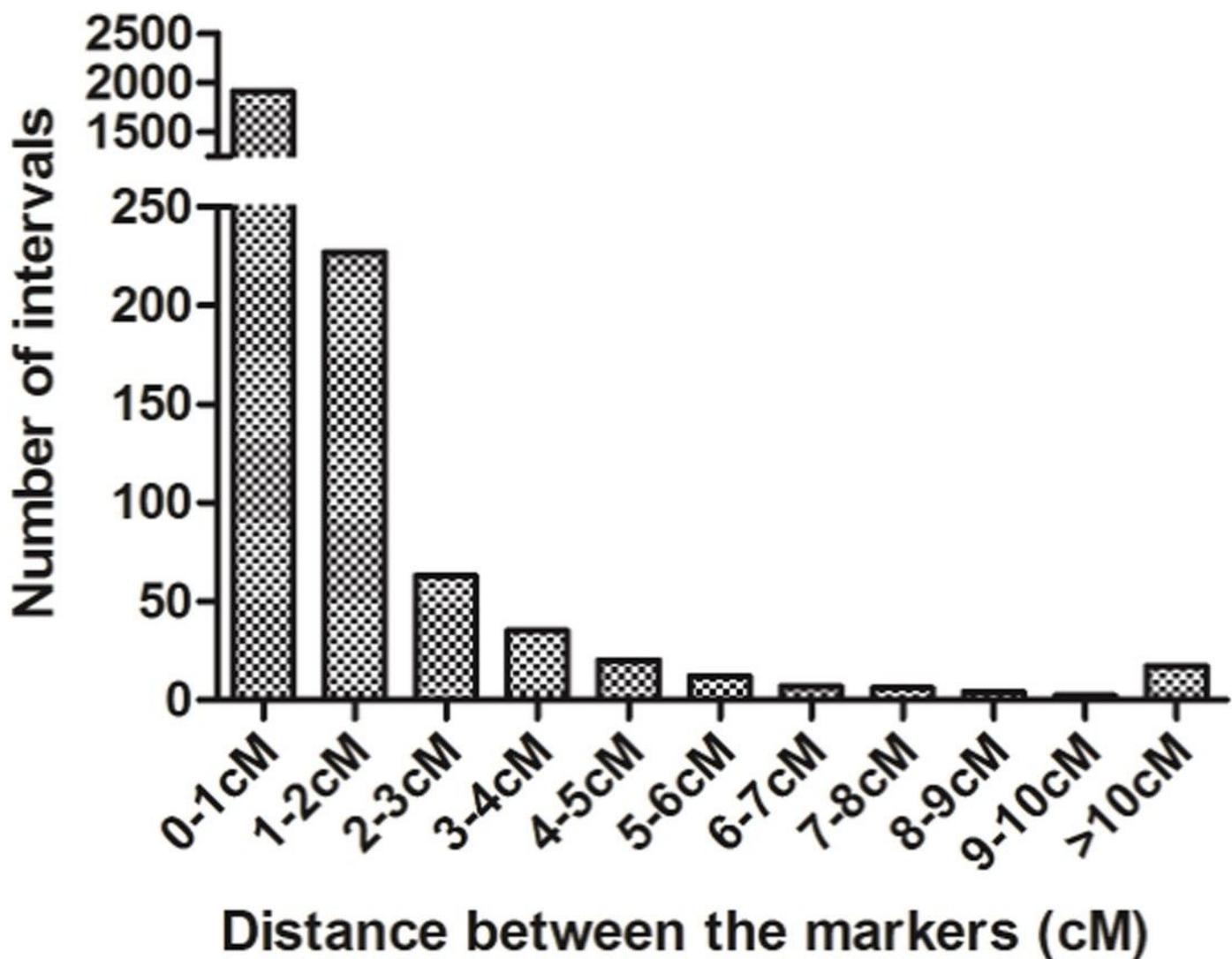


Figure 2

Distribution of map distance between adjacent markers in sex-averaged genetic map of *S. chuatsi*.

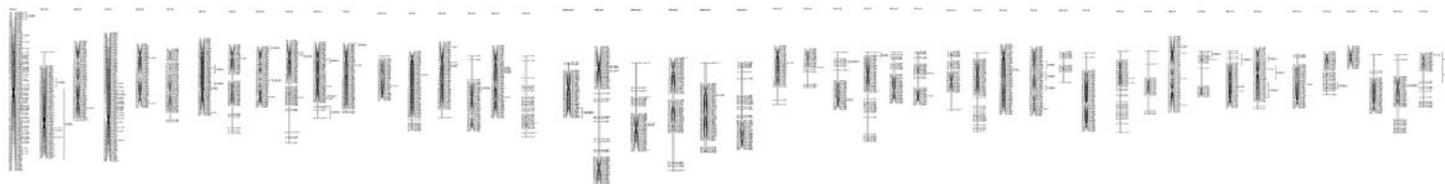


Figure 3

Sex-specific maps containing SDL of *S. chuatsi* based on SNPs. The male linkage group (left) is named “MSD-LG1-MSD-LG24”; the female linkage group (right) is named “FSD-LG1-FSD-LG24”. Total lengths of linkage groups are expressed as Kosambi cM. The statistical significance of markers with segregation-distortion was

indicated with stars, with $P < 0.05 = *$; $P < 0.01 = **$; $P < 0.005 = ***$ or more stars. MSDR: segregation-distortion region in male map; FSDR: segregation-distortion region in female map.