Development and Characterization of 35 SNP Markers in the Fat Greenling *Hexagrammos Otakii*

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Abstract

Hexagrammos otakii is an important kind of economic fish species in East Asia. However, wild *H. otakii* resources have declined sharply in recent years as a result of human disturbance and habitat destruction. Thus, it is crucial to protect the current resources of *H. otakii*. In this study, 35 novel single nucleotide polymorphism (SNP) markers were developed based on restriction-site associated DNA sequencing. The results showed that the observed heterozygosity and expected heterozygosity ranged from 0.1875 to 0.6562 and 0.2679 to 0.5079, respectively. The minor allele frequency ranged from 0.1875 to 0.4375. Polymorphic information content ranged from 0.229 to 0.375. Six SNPs were found to be deviated significantly from the HWE ($P < 0.05$). These SNP markers will serve as a useful tool for genetic studies and population evaluation aimed at the conservation of *H. otakii*.

Full Text

Hexagrammos otakii (Fat greenling), belonging to family Hexagrammidae and genus *Hexagrammos*, mainly distributed in the North Korean, Japan and China offshore (Nishikawa 1982). *H. otakii* is an important kind of economic fish species in East Asia. *H. otakii* has high protein content (18.50%) and essential amino-acid (7.25%), especially the protein content is higher than most other fish (Kang and Wu 1999). Featuring meat quality, good taste, high nutritional value, *H. otakii* is challenged by continuously growing market requirement and reduction of fishery resources (Shen et al. 2019). Thus, demand for hypervariable molecular markers to provide a population-genetic perspective on conservation and management efforts of the species (FAO 1993) becomes urgent.

With the rapid development of next-generation sequencing technologies (NGS) (Davey et al. 2011), single nucleotide polymorphisms (SNPs) have been largely developed and widely used for genetic studies in aquaculture species such as *Sebastes schlegelii* (Gao et al. 2021), *Megalobrama terminalis* (Yang et al. 2020) and *Coilia ectenes* (Yu et al. 2019). For *H. otakii* resource conservation, we implemented restriction-site associated DNA (RAD) sequencing to facilitate the genetic evaluation.

In this study, a total of 32 *H. otakii* wild individuals were collected from northern Yellow Sea in China. Muscle tissues were sampled and stored in 95% molecular grade ethanol. Total genomic DNA was extracted from tissue samples using the TIANamp Marine Animals DNA Kit (Tiangen, Beijing, China) following the manufacturer's instructions. All samples were used to construct the RAD libraries. Then, the libraries were sequenced on the Illumina HiSeq 4000 platform using 150 base pair (bp) paired-end reads. We trimmed the adapter sequences and low-quality reads (Phred score < 20) with Cutadapt (Martin 2011). Finally, 707,105 putative SNPs with the highest scores were generated, from which we randomly selected 100 candidate SNPs to test their applicability. The polymorphism of these candidate SNPs was further characterized in the samples mentioned above. Primer sequences for SNP loci were designed by Primer 5.0 software. The PCR reactions were conducted in 25 μ L volume containing 50 ng of genomic DNA, 1 $\times$ PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 250 nM of each primer, and 1U of *Taq* polymerase (Takara, Dalian, China). The amplicons were checked by 1.0 % agarose gel electrophoresis and sequenced on ABI 3730 DNA Analyzer (Applied Biosystems).

The observed heterozygosity (H_o), expected heterozygosity (H_e), minor allele frequency (MAF), and P value representing the deviations from the Hardy-Weinberg equilibrium were estimated using POPGENE 32 (Yeh et al. 2000). The polymorphism information content (PIC) was calculated using Cervus 3.0 (Kalinowski et al. 2007). Among the test SNP markers in *H. otakii*, 35 polymorphic SNP markers were characterized in Table 1. The H_o and H_e were ranged from 0.1875 to 0.6562 and 0.2679 to 0.5079, respectively. The MAF ranged from 0.1875 to 0.4375. The PIC varied from 0.229 to 0.375, with an average of 0.3293. Six SNPs were found to be deviated significantly from the HWE ($P < 0.05$). These results will be useful for understanding the genetic diversity of *H. otakii* to assist in the management of this germplasm resource.

Declarations

Author contributions G.X.G. conceived and designed the study, contributed to the drafted the manuscript. L.W.D. contributed to the analysis and writing. S.W. and L.Y.L. participated in the data collection, purification, and management of the samples for sequencing and genotyping. B.X.B. contributed to the design of the study and writing. L.Z.Y. and L.Y.F. contributed to the collection of the samples. All authors have reviewed and approved the manuscript.

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Availability of data The sequence data used for SNP discovery belongs to Liaoning Ocean and Fisheries Science Institute, and it can be available upon reasonable request.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Investigations were conducted according to the guiding principles for the use and care of laboratory animals and in compliance with the Academic Ethics Committee of Liaoning Ocean and Fisheries Science Institute. Our study had been submitted to and approved by the Academic Ethics Committee of Liaoning Ocean and Fisheries Science Institute. The research permits also included the necessary ethical approval in terms of sample collection. All sample collection was undertaken in accordance with relevant Academic Ethics Committee of Liaoning Ocean and Fisheries Science Institute guidelines and regulations.

Consent to participate Not applicable

Consent for publish Not applicable

References

1. Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nat Rev Genet* 12(7):499–510. <https://doi.org/10.1038/nrg3012>
2. FAO (1993) Report of the expert consultation on utilization and conservation of aquatic genetic resources. *FAO Fish Rep* 491:1–58
3. Gao XG, Bao XB, Sun W, Li YF, Liu ZY, Liu WD (2021) Isolation and characterization of 38 SNP markers for the black rockfish, *Sebastes schlegelii* by nextgeneration sequencing. *Conserv Genet Resour.* <https://doi.org/10.1007/s12686-021-01226-3>
4. Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 16:1099–1106
5. Kang B, Wu YF (1999) Nutritional composition analyses of *Hexagrammos otakii* marine. *Science* 1:23–25
6. Martin M (2011) Cutadapt removes adapter sequences from high throughput sequencing reads. *EMBnet J* 17(1):10–12
7. Nishikawa SKS (1982) The karyotype of a hexagrammid, *Hexagrammos otakii* (Pisces, Scorpaeniformes). *Chromosome Info* 33:16–17
8. Shen Z, Wang B, Hu F, Liu H, Guan H, Zheng F (2019) Isolation and characterization of microsatellite loci in *Hexagrammos otakii* based on 2b-RAD method. *J Appl Ichthyol* 36(1):55–61. <https://doi.org/10.1111/jai.13988>
9. Yang JP, Li XH, Li YF, Zhu SL, Chen WT, Li J (2020) Isolation and characterization of 30 SNP markers in Guangdong bream (*Megalobrama terminalis*) by next-generation sequencing. *Conserv Genet Resour.*

<https://doi.org/10.1007/s12686-020-01131-1>

10. Yeh FC, Yang R, Boyle TJ, Ye Z, Xiyan JM (2000) POPGENE 32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Microsoft Windows-based freeware for population genetic analysis
11. Yu AQ, Shi YH, Yan YL (2019) Development and characterization of 50 snp markers in *Coilia ectenes*. Conserv Genet Resour. [https:// doi.org/10.1007/s12686-019-01086-y](https://doi.org/10.1007/s12686-019-01086-y)

Tables

Table 1
Summary information for the 35 SNP markers developed for the *H. otakii*

Primer ID	Primer sequences	SNP type	SNP Position	Ho	He	MAF	PIC	P _{HWE}
SNP1	F:GTGCCGATGTTTATATCAGGCG R:cacatttctggttcgggctc	AT	97	0.3750	0.5060	0.3125	0.374	0.1368
SNP2	F:AATTCTGGTCCGGCAACAA R:agcccagatgaacagagac	AG	118	0.3125	0.3095	0.3125	0.271	0.9550
SNP3	F:TGCAGGTGAACATAGATTTCAG R:tgctctctgatgatggca	AG	104	0.2188	0.2892	0.2188	0.244	0.1510
SNP4	F:TCTTGTCCCTCCACTGTTGC R:tccggtcacaactccacc	AG	95	0.2500	0.3472	0.2500	0.283	0.1022
SNP5	F:TCAAACAGGGAGAAACGGC R:Ttaccactgaagagactccc	AG	73	0.5938	0.5074	0.1875	0.375	0.3283
SNP6	F:GGAACGTAGGGGAGTTGAGC R:CACGCACATACGCACATACG	TG	86	0.2500	0.2679	0.2500	0.229	0.6929
SNP7	F:TTCCCAGGTAAAGCCAAGC R:aatacctcctcgctactcg	AC	106	0.5312	0.4479	0.4063	0.349	0.2828
SNP8	F:AAGTGTTTCATCCGCTGGCTTA R:tcattgtaggtagccacacg	AC	90	0.6562	0.4836	0.2813	0.363	0.0400*
SNP9	F:GTAAACTGAATATCAACAGGTG R:aactttgtaaagtgtcctgg	TG	67	0.3750	0.3810	0.375	0.305	0.9277
SNP10	F:TGTTTGTGTTGCCACTATCAG R:GCTcaatgtcttgaactggg	AC	64	0.4688	0.5035	0.3125	0.373	0.6917
SNP11	F:TGTTAACGTTTCGGCCTGCTAG R:agcaggaagtcaaccacg	AC	76	0.4375	0.3472	0.4375	0.283	0.1291
SNP12	F:TTCTCTGTTACAGCAGCGC R:caatgatagcacaggcagc	AG	95	0.4688	0.5074	0.2500	0.374	0.6612
SNP13	F:TCTGACTGACATCGTGGATCAC R:atgttctcaggttgcacagg	CT	70	0.2500	0.4365	0.2500	0.337	0.0136*
SNP14	F:CGTAGGCTGTTGCGTCTC R:Tgtgcacaagtataggctgc	CT	78	0.2188	0.4241	0.2188	0.330	0.0051*
SNP15	F:ATTCAGCAGTGGACTTGGGG R:TTCACCTCTTCCCGGGCTTC	CT	118	0.1875	0.3095	0.1875	0.258	0.0206*
SNP16	F:TCTACTATGACGCTGTTACTCCAG R:atggtcaagtcacagtggc	AG	83	0.2812	0.3289	0.2813	0.271	0.3967
SNP17	F:ACACGATACTGCTTTGTCAC R:Gcttctagattagcttagcgc	CT	81	0.3125	0.3810	0.2813	0.305	0.2964
SNP18	F:ATGGATTTGCAGCACGGTTG R:AGCTAAACTGGCAGCTCA	AG	102	0.3750	0.5060	0.2500	0.374	0.1368
SNP19	F:CACTGGTGTGTTTGTGTCAAGG R:tccagcaccatcatcaggtc	TG	67	0.3438	0.5074	0.3438	0.375	0.0637
SNP20	F:AAGAGAGAAAATACCAACACTG R:aaatgatcctggctcctgc	AG	70	0.5312	0.5035	0.2500	0.373	0.7511

Primer ID	Primer sequences	SNP type	SNP Position	Ho	He	MAF	PIC	P _{HWE}
SNP21	F:CGTTGGTTGTTAAGTGGCAG R:caactaaggcagaagagatagacc	AG	64	0.5000	0.5079	0.2500	0.375	0.9284
SNP22	F:TTGGTTTTTGACGGTGTTCGC R:GGTGGAGTGGCTGTGATTCT	CT	99	0.4375	0.5079	0.2500	0.375	0.4255
SNP23	F:AGAGTGCTGAAGCCTCAACTG R:acgtgatggaaagggacactg	TG	70	0.2812	0.3289	0.2813	0.271	0.3967
SNP24	F:TTCGGTTACGCGGGAGTTTGTC R:gtggaacccacaggtaacgtc	CT	117	0.4062	0.4836	0.4063	0.363	0.3570
SNP25	F:AGAGTCAAGACTAAGCGCG R:ctgtgtgagttttggtggc	CT	102	0.3438	0.4479	0.3438	0.344	0.1794
SNP26	F:CTGCCGTGACGAGTTCAGAT R:TGTGCGATCGTGTGACAGAA	CT	71	0.4688	0.4678	0.4063	0.354	0.9902
SNP27	F:CATAACGGACAAGAAAAATGCCTC R:ttaaccagtgactgtctggac	CT	74	0.5625	0.4762	0.3438	0.359	0.2965
SNP28	F:ACCGTGTGCTTTCAGTTCCTG R:Gtaacactggagggtggagc	CT	100	0.5312	0.4678	0.3750	0.354	0.4340
SNP29	F:GCCACATTCTACACACTGTCCC R:tcagcagacgacaatgcagc	CT	75	0.3438	0.4479	0.3438	0.344	0.1794
SNP30	F:TCCTTCTCTGGAGTCTTTGTGC R:TGTGTTTTGAGTTTTTGCAAGC	CT	74	0.3750	0.4107	0.3750	0.330	0.6145
SNP31	F:CTCTCCAAGCCTCAGCGAG R:ACTTCCTCTCCTCCACTGCA	AG	113	0.5000	0.4901	0.3125	0.366	0.9073
SNP32	F:CAGGTCTCAAGCTCACCCCTGG R:actactgcggatcacttgttgc	TG	94	0.3125	0.3095	0.3125	0.258	0.9550
SNP33	F:AGCATTGCTTGATAATGACTGCC R:agcgaatatctgcgaaacgg	AT	98	0.5000	0.5079	0.2188	0.375	0.9284
SNP34	F:GCTGCCACAGTCACAGTTTCAC R:tcaggtcctcagcatcaacgg	AG	72	0.2500	0.4583	0.2500	0.349	0.0087*
SNP35	F:AGATGCAAGAGGGCCACTTCC R:agggatagaggctgacgcctg	CT	103	0.1875	0.3095	0.1875	0.258	0.0206*

Ho observed heterozygosity, He expected heterozygosity, MAF minor allele frequency, PIC polymorphism information content, P_{HWE} the P value of the deviations from the Hardy–Weinberg equilibrium

*P<0.05