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# 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*.

## Keywords

*Hexagrammos otakii*, SNP, RAD, Genetic Diversity

## 1. Introduction

*Hexagrammos otakii* (Fat greenling), belonging to the family Hexagrammidae and genus *Hexagrammos*, is mainly distributed in the North Korea, Japan and China offshore [1]. *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 since the protein content is higher than most other fish [2]. Featuring meat quality, good taste, and high nutritional value, *H. otakii* is chal-

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lenged by continuously growing market requirements and reduction of fishery resources [3]. Thus, the demand for hypervariable molecular markers to provide a population-genetic perspective on conservation and management efforts of the spices becomes urgent [4] [5].

With the rapid development of next-generation sequencing technologies (NGS) [6], single nucleotide polymorphisms (SNPs) have been largely developed and widely used for genetic studies in aquaculture species such as *Sebastes schlegelii*, *Megalobrama terminalis* and *Coilia ectenes* [7] [8] [9]. For *H. otakii* resource conservation, we implemented restriction-site associated DNA (RAD) sequencing to facilitate the genetic evaluation which could provide a reference for the development of SNP markers.

## 2. Materials and Methods

### 2.1. Materials

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.

### 2.2. Methods

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 [10]. 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 μL volume containing 50 ng of genomic DNA, 1 × 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).

## 3. Results

The observed heterozygosity (*Ho*), expected heterozygosity (*He*), minor allele frequency (MAF), and P value representing the deviations from the Hardy-Weinberg equilibrium were estimated using POPGENE 32. The polymorphism information content (PIC) was calculated using Cervus 3.0 [11]. Among the test SNP markers in *H. otakii*, 35 polymorphic SNP markers were characterized in **Table 1**. The *Ho* and *He* 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 de-

viated significantly from the HWE ($P < 0.05$) (see Table 2 for details). These results will be useful for understanding the genetic diversity of *H. otakii* to assist in the management of this germplasm resource.

**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<br>R: CACATTTCTGGTTCGGGCTC | AT | 97 | 0.3750 | 0.5060 | 0.3125 | 0.374 | 0.1368 |
| SNP2 | F: AATTCTGGTCCGGCAACAA<br>R: AGCCCGAGATGAACAGAGAC | AG | 118 | 0.3125 | 0.3095 | 0.3125 | 0.271 | 0.9550 |
| SNP3 | F: TGCAGGTGAACATAGATTTCCAG<br>R: TGCTCCTCTGATGATGGCA | AG | 104 | 0.2188 | 0.2892 | 0.2188 | 0.244 | 0.1510 |
| SNP4 | F: TCTTGTCCCTCCACTGTTGC<br>R: TCCGGTCACAACTTCCACC | AG | 95 | 0.2500 | 0.3472 | 0.2500 | 0.283 | 0.1022 |
| SNP5 | F: TCAAACAGGGAGAAACGGC<br>R: TTACCACTTGAAGAGACTCCC | AG | 73 | 0.5938 | 0.5074 | 0.1875 | 0.375 | 0.3283 |
| SNP6 | F: GGAACGTAGGGGAGTTGAGC<br>R: CACGCACATACGCACATACG | TG | 86 | 0.2500 | 0.2679 | 0.2500 | 0.229 | 0.6929 |
| SNP7 | F: TTCCCAGGTAAAGCCAAGC<br>R: AATACCTCCTCGCTACTCG | AC | 106 | 0.5312 | 0.4479 | 0.4063 | 0.349 | 0.2828 |
| SNP8 | F: AAGTGTTCATCCGCTGGCTTA<br>R: TCATTGTAGGTAGCCCACACG | AC | 90 | 0.6562 | 0.4836 | 0.2813 | 0.363 | 0.0400* |
| SNP9 | F: GTTAAACTGAATATCAACAGGTG<br>R: AACTTTGTAAAGTGTCCTGG | TG | 67 | 0.3750 | 0.3810 | 0.375 | 0.305 | 0.9277 |
| SNP10 | F: TGTTTGTGTTGCCACTATCAG<br>R: GCTCAATGTCTTGAACTGGG | AC | 64 | 0.4688 | 0.5035 | 0.3125 | 0.373 | 0.6917 |
| SNP11 | F: TGTTAACGTTTCGGCCTGCTAG<br>R: AGCAGGAAGTCAACCCACG | AC | 76 | 0.4375 | 0.3472 | 0.4375 | 0.283 | 0.1291 |
| SNP12 | F: TTCTCTGTTACAGCAGCGC<br>R: CAATGATAGCACAGGCAGC | AG | 95 | 0.4688 | 0.5074 | 0.2500 | 0.374 | 0.6612 |
| SNP13 | F: TCTGACTGACATCGTGGATCAC<br>R: ATGTTCTCAGGTTGCATCAGG | CT | 70 | 0.2500 | 0.4365 | 0.2500 | 0.337 | 0.0136* |
| SNP14 | F: CGTAGGCTGTTGCGTCTC<br>R: TGTGCACAAGTATAGGCTGC | CT | 78 | 0.2188 | 0.4241 | 0.2188 | 0.330 | 0.0051* |
| SNP15 | F: ATTCAGCAGTGGACTTGGGG<br>R: TTCACTCTTTCCCGGGCTTC | CT | 118 | 0.1875 | 0.3095 | 0.1875 | 0.258 | 0.0206* |
| SNP16 | F: TCTACTATGACGCTGTTACTCCAG<br>R: ATGGTCAAGTCATCAGTGGC | AG | 83 | 0.2812 | 0.3289 | 0.2813 | 0.271 | 0.3967 |
| SNP17 | F: ACACGATACTGCTTTGTCAC<br>R: GCTTCTAGATTAGCTTAGCGC | CT | 81 | 0.3125 | 0.3810 | 0.2813 | 0.305 | 0.2964 |
| SNP18 | F: ATGGATTTGCAGCACGGTTG<br>R: AGCTAAAACTGGCACGCTCA | AG | 102 | 0.3750 | 0.5060 | 0.2500 | 0.374 | 0.1368 |
| SNP19 | F: CACTGGTGTGTTTGTGTCAAGG<br>R: TCCAGCACCATCATCAGGTC | TG | 67 | 0.3438 | 0.5074 | 0.3438 | 0.375 | 0.0637 |

**Continued**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| SNP20 | F: AAGAGAGAAAATACCAACACTG<br>R: AAATGATCCTGGTCCTTGC | AG | 70 | 0.5312 | 0.5035 | 0.2500 | 0.373 | 0.7511 |
| SNP21 | F: CGTTGGTTGTTAAGTGGCAG<br>R: CAACTAAGGCAGAAGAGATAGACC | AG | 64 | 0.5000 | 0.5079 | 0.2500 | 0.375 | 0.9284 |
| SNP22 | F: TTGGTTTTTGACGGTGTCGC<br>R: GGTGGAGTGGCTGTGATTCT | CT | 99 | 0.4375 | 0.5079 | 0.2500 | 0.375 | 0.4255 |
| SNP23 | F: AGAGTGCTGAAGCCTCAACTG<br>R: ACGTGATGGAAAGGGACACTG | TG | 70 | 0.2812 | 0.3289 | 0.2813 | 0.271 | 0.3967 |
| SNP24 | F: TTCGGTTACGCGGGAGTTTGTC<br>R: GTGGAACCCACAGGGTAACGTC | CT | 117 | 0.4062 | 0.4836 | 0.4063 | 0.363 | 0.3570 |
| SNP25 | F: AGAGTCAAGACTAAGCGCG<br>R: CTGTGTGAGTTTTTGGTGGC | CT | 102 | 0.3438 | 0.4479 | 0.3438 | 0.344 | 0.1794 |
| SNP26 | F: CTGCCGTGACGAGTTCAGAT<br>R: TGTGCGATCGTGTGACAGAA | CT | 71 | 0.4688 | 0.4678 | 0.4063 | 0.354 | 0.9902 |
| SNP27 | F: CATAACGGACAAGAAAAATGCCTC<br>R: TTAACCAGTGACTGTCTGGAC | CT | 74 | 0.5625 | 0.4762 | 0.3438 | 0.359 | 0.2965 |
| SNP28 | F: ACCGTGTGCTTTCAGTTCCTG<br>R: GTAACACTGGAGGGTGGAGC | CT | 100 | 0.5312 | 0.4678 | 0.3750 | 0.354 | 0.4340 |
| SNP29 | F: GCCACATTCTACACACTGTCCC<br>R: TCAGCAGACGACAATGCAGC | CT | 75 | 0.3438 | 0.4479 | 0.3438 | 0.344 | 0.1794 |
| SNP30 | F: TCCTTCTCTGGAGTCTTTGTGC<br>R: TGTGTTTTGAGGTTTTTGCAAGC | CT | 74 | 0.3750 | 0.4107 | 0.3750 | 0.330 | 0.6145 |
| SNP31 | F: CTCTCCAAGCCTCAGCGAG<br>R: ACTTCCTCTCCTCCACTGCA | AG | 113 | 0.5000 | 0.4901 | 0.3125 | 0.366 | 0.9073 |
| SNP32 | F: CAGGTCTCAAGCTCACCCTGG<br>R: ACTACTTGCGGATCACTTGTTGCC | TG | 94 | 0.3125 | 0.3095 | 0.3125 | 0.258 | 0.9550 |
| SNP33 | F: AGCATTGCTTGATAATGACTGCC<br>R: AGCGAATATCTGCGAAACGG | AT | 98 | 0.5000 | 0.5079 | 0.2188 | 0.375 | 0.9284 |
| SNP34 | F: GCTGCCACAGTCACAGTTTCAC<br>R: TCAGGTCCTCAGCATCAACGG | AG | 72 | 0.2500 | 0.4583 | 0.2500 | 0.349 | 0.0087* |
| SNP35 | F: AGATGCAAGAGGGCCACTTCC<br>R: AGGGATAGAGGCTGACGCCTG | CT | 103 | 0.1875 | 0.3095 | 0.1875 | 0.258 | 0.0206* |

**Table 2.** The detailed information about the six SNPs found to be deviated significantly from the HWE ($P < 0.05$).

| Primer ID | Genotype/observed value | Genotype/expected value | $\chi^2$ ($P_{HWE}$) |
|---|---|---|---|
| SNP8 | AA/9, AC/21, CC/2 | AA/11.88, AC/15.23, CC/4.88 | 4.58 (0.0400) |
| SNP13 | CC/18, CT/8, TT/6 | CC/15.13, CT/13.75, TT/3.13 | 5.60 (0.0136) |
| SNP14 | TT/19, C/T7, CC/6 | TT/15.82, CT/13.36, CC/2.82 | 7.26 (0.0051) |
| SNP15 | TT/23, CT/6, CC/3 | TT/21.13, CT/9.75, CC/1.13 | 4.72 (0.0206) |
| SNP34 | GG/17, GA/8, AA/7 | GG/13.78, GA/14.44, AA/3.78 | 6.39 (0.0087) |
| SNP35 | TT/23, CT/6, CC/3 | TT/21.13, CT/9.75, CC/1.13 | 4.72 (0.0206) |

## 4. Conclusion

Wild *H. otakii* resources have declined sharply in recent years as a result of human disturbance and habitat destruction. In this study, 35 novel SNP markers were developed based on restriction-site associated DNA sequencing. The results showed that the *Ho* and *He* 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*.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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