Microsatellite Analysis of Genetic Variation in Intraspecific Hybrids and Self-Breds of Steelhead Trout and Golden Rainbow Trout

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Abstract. Genetic diversity of the hybrids and inbreds of Steelhead trout and golden rainbow trout, altogether four populations, was studied based on 15 polymorphic microsatellite locus. Two hundred and forty individuals (sixty individuals per one population) were collected from the Yudu hill aquaculture experimental station of Beijing Fisheries Research Institute, Beijing, China. The genetic variation of the hybrids and inbreds of Steelhead trout and golden rainbow trout were all relatively high. The mean observed heterozygosity value of YJ, YY, JJ and JY were respectively 0.6600, 0.7733, 0.7822, and 0.6533. The mean Fis and Fit value were respectively (-0.5065, -0.6037, -0.5722, -0.4620) and (-0.3857, -0.5272, -0.4987, -0.4069), showing an average excess of heterozygote proportion. The mean Fst value was respectively 0.0802, 0.0477, 0.0471, and 0.0376. The mean genetic distance between JJ and YY was the closest (0.0097), next to YJ and YY (0.0655), and that between JY and YY was the most distant (0.1096). The dendrogram showed JY and YJ firstly clustered together, and then they clustered together with the inbred offspring (JJ and YY). Due to Fst (little genetic differentiation) and Nm (the Nm values of 15 locus > 1), JY was a finer variety the two hybrids of Steelhead trout and golden rainbow trout. Our data offer supporting evidence that inbreeding depression of Steelhead trout and golden rainbow trout involves multiple related genes, favoring partial dominance over dominance.

1. Introduction
The range of coastal rainbow trout was Pacific Ocean tributaries from Aleutian Islands in Alaska south to Southern California. Anadromous forms were known as steelhead, freshwater forms as rainbow trout. The steelhead trout was an anadromous (sea-run) form of the coastal rainbow trout (Oncorhynchus mykiss irideus) that usually returns to fresh water to spawn after living two to three years in the ocean. Because of the contribution of genetic engineering and domestication means of improvement, Steelhead could have been cultured intensive in fresh water. Steelhead was introduced from America to China in 1998, and began to be cultured. Golden rainbow trout or palomino trout were bred from a single mutated color variant of Oncorhynchus mykiss that originated in a West Virginia fish hatchery in 1955 [1]. Golden rainbow trout was predominantly yellowish, lacking the typical green field and black spots but retaining the diffuse red stripe. The palomino trout was a mix of golden and common rainbow trout, resulting in an intermediate color. Golden rainbow trout was introduced from Japan to China in 1996.

Hybridization is widely used to increase growth rate, manipulate sex ratios, and produce sterile fish and to improve flesh quality, disease resistance and environmental tolerance in aquaculture [2-6]. Although inbreeding occurs naturally, much of the evidence for inbreeding stems from direct or
indirect results of human activity. The potential consequences of inbreeding highlight the importance of maintaining genetic diversity in salmonid populations. Rainbow trout, primarily hatchery-raised fish of the coastal rainbow trout subspecies (*Oncorhynchus mykiss irideus*) introduced into waters inhabited with cutthroat trout, will breed with cutthroats and produce fertile hybrids called cutbows [7] In the case of the westslope cutthroat trout (*Oncorhynchus clarki lewisi*), hybridization with introduced rainbow and Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) is threatening the westslope cutthroat trout with genomic extinction. Such introductions into the ranges of redband trout (*Oncorhynchus mykiss gairdneri*, newberrii, and stonei) have severely reduced the range of pure stocks of these subspecies, making them “species of concern” in their respective ranges [8]. Within the range of the Kern River golden trout of Southern California, hatchery-bred rainbows introduced into the Kern River have diluted the genetic purity of the Kern River rainbow trout (*Oncorhynchus mykiss gilberti*) and golden trout (*Oncorhynchus mykiss aguabonita*) through intraspecific breeding [9,10]. Genetic interactions between cultured and wild salmonids had allowed widespread practices that can reduce genetic variability in natural populations. Although studies have detected inbreeding depression in wild, anadromous salmonids, its genetic basis has rarely been addressed in cultured, landlocked salmon of aquaculture farms.

Because of highly polymorphic, co-dominant, microsatellite DNA markers had high discriminatory power. The highly polymorphic, randomly distributed microsatellite loci makes these markers particularly suited for the investigation of kinship relationships [11], population genetics and genome mapping [12]. The aim of the present study was to assess the genetic diversity and genetic variability among the intraspecific hybrids and self-breds of steelhead trout and golden rainbow trout, and to investigate the cluster result and the mechanism of inbreeding depression.

2. Materials and Methods

2.1. Sample Collection
From January, 2014 to July, 2014, the intraspecific hybrid and self-bred progeny by a method of artificial fertilization in the Yudu hill aquaculture experimental station of Beijing Fisheries Research Institute, Beijing, China. 24 steelhead trout (16♀, 8♂) was introduced from USA at 2007, and 24 golden rainbow trout (16♀, 8♂) was introduced from Japan at 2005. The progeny of the two intraspecific hybrid and self-bred groups were cultured in the separate tanks until they were 50g. At last, a total of 72 000 offspring were obtained: steelhead trout, 21 000, (steelhead trout♀× golden rainbow trout♂),15 000, (golden rainbow trout♀× steelhead trout♂),17 000, golden rainbow trout, 19 000. Samples were selected randomly (i) 30 self-bred progeny from 21000 steelhead trout, (ii) 30 intraspecific hybrid progeny from 15 000(steelhead trout♀× golden rainbow trout♂), (iii) 30 intraspecific hybrid progenys from 17 000(steelhead trout♀× golden rainbow trout♂) and (iv) 30 self-bred progeny from 19 000 golden rainbow trout.

2.2. Genomic DNA Extraction and PCR Amplification
Fin clips of 120 individuals were collected. DNA was isolated with DNAiso Reagent (TaKaRa, China). The concentration of each DNA sample was estimated by spectrophotometry and diluted to a working concentration of 10 pg/μL for use in polymerase chain reaction (PCR). In this study, SSR sequences were obtained from the DNA sequences of GenBank by SSR Hunter. Then 15 primers (Table 1), were designed by Primer 5 with the SSR sequence, and were selected for PCR. Amplifications were performed in a 50μL reaction volume consisting of Prime STAR® HS DNA Polymerase (TaKaRa, China), containing 10 μL of 5×PrimeSTAR Buffer (Mg²⁺ Plus), 4μL of 10 mmol/L dNTP, 1μL of 10μM Primer1 and Primer2, 1 μL of 10 pg/μL genomic DNA, 0.5 μL of 2.5 U/μL PrimeSTAR® HS DNA Polymerase. Microsatellite amplifications were performed in the thermal cycling (Biometra) with the following protocol: 30cycles of 10 s at 94°C, 15s at annealing temperature and 30 s at 72°C, and a final extension at 72 °C for 10 min. The PCR amplification products were detected using the ABI Prism 3730XL genetic analyzer, and the data was collected and analyzed by data collection software and genemapper software.
Table 1. Primer sequences, annealing temperature, and allele size for molecular markers used in DNA analyses

| Primer | Sequence 5'-3' | Anneal temp (°C) | Allele size (bp) |
|--------|----------------|------------------|-----------------|
| 1 BJY9  | TAGTTCCCCCTCATAACCC AGGCCAAGACCCACTACGC | 59.5 | A, 140; B, 248; C, 442 |
| 2 BJY11 | TGGAGCAGTGTGACTGCTGCTT TGGGTTGTTCTTTTGT | 51.2 | A, 196; B, 397; C, 472 |
| 3 BJY17 | CCCCTATTCTTTCCTCACA AGGCGGAAATGCGCAAC | 57.5 | A, 178; B, 370; C, 464 |
| 4 BJY18 | TCCCTGGCTTCAAGTTAGG TGGACCCCTGGAACGTAGTAT | 59.6 | A, 114; B, 188 |
| 5 BHW5  | AGGCTTATCAGGGGTTTC CTTGAAGGGAATAAGT | 48.1 | A, 106; B, 259; C, 382 |
| 6 BHW6  | TACAGGCTGGATAAGTT GCGGTTAAAGGGAAG | 51.0 | A, 96; B, 175; C, 340 |
| 7 BHW10 | CACCTTACCTTTAGCAC AGCAAGCCATTTAGTTC | 41.8 | A, 72; B, 169; C, 294 |
| 8 BHW12 | TAGCTAGTGAAGCTTGGGTC AGTGGATGGTGCTGATGT | 44.4 | A, 175; B, 146; C, 214 |
| 9 BHW13 | TGTCTTTGGCTCTTTTC | 43.2 | A, 162; B, 168; C, 213; D, 174; E, 192 |
| 10 BHW14 | GACCTTGAGTGTGAGCTTC CCCTTACCCCTAAGAC | 44.7 | A, 88; B, 192; C, 333 |
| 11 BHW16 | AGTCTGGCGTGTTTGGAGTG GAGACGGAAAGAGGGTG | 44.7 | A, 83; B, 172; C, 214 |
| 12 BHW17 | AGAAGGAGAATCAGAGGAAGG TCAAGCCAATTGAGGAAAC | 42.2 | A, 116; B, 188 |
| 13 BHW19 | GAGATGATGAGAAGGTAG | 45.0 | A, 100; B, 136; C, 192; D, 213 |
| 14 BHW20 | ACTGGAATAGTTAGCGAGGT TACCCCAACACCACTCCAG | 42.2 | A, 111; B, 161; C, 378 |
| 15 BHW12 | TATGGGAGACTTTGAGGCC GATTGGAATAGGGCAGCT | 45.9 | A, 143; B, 210; C, 450 |

2.3. Genetic Diversity and Inheritance Characteristics of Loci
The Observed number of alleles (Na). Effective number of alleles (Ne) [13], Shannon’s Information index (I) [14], observed homozygositys (Obs_Hom), expected homozygositys (Exp_Hom) [15], observed heterozygosities (Obs_Het), expected heterozygosities (Exp_Het), fixation index (Fis: subpopulation inbreeding coefficient, Fit: overall inbreeding coefficient, Fst: degree of genetic differentiation of subpopulations) [16], genetic distances, genetic similarity were calculated for each locus using POPGENE 32. Furthermore, distances between pairs of individuals based on allele sharing were calculated using MEGA 4.0 of unweighted pair group method using arithmetic mean (UPGMA).

3. Results
3.1. Allelic Variation and Homozygosity
15 pairs of microsatellites markers (primer sequences listed in Table 1) with highly polymorphic were detected in intraspecific hybrids and self-bred of Steelhead trout and golden rainbow trout, and a total of 46 alleles were amplified. For YJ, YY, JJ and YJ, the mean value of na (Observed number of alleles) of 15 locus were respectively 2.8667, 2.8000, 2.7333, and 3.0000, and the mean value of ne (Effective number of alleles) were respectively 2.0334, 2.1135, 2.2263, 1.9842. For YJ, YY, JJ and YJ, the mean value of I (Shannon’s Information index) of 15 locus were respectively 0.7789, 0.8240, 0.8448, 0.7569, and there was no difference significantly between any two of them (listed in Table 2). Obviously, the
above-mentioned data showed a relatively higher genetic diversity in YJ, YY, JJ and JY. For YJ, YY, JJ and JY, the mean observed homozygosty value of 15 locus (respectively 0.3400, 0.2267, 0.2178, 0.3467) were lower than the mean expected homozygosty value (respectively 0.5156,0.4762,0.4601,0.5278 ) (listed in Table 3).

| Name | Locus | Sample Size | na*   | ne*   | I*    |
|------|-------|-------------|-------|-------|-------|
| YJ   | Mean  | 60          | 2.8667| 2.0334| 0.7789|
|      | St.   | Dev         | 0.5164| 0.4990| 0.2290|
| YY   | Mean  | 60          | 2.8000| 2.1135| 0.8240|
|      | St.   | Dev         | 0.6761| 0.4454| 0.2028|
| JJ   | Mean  | 60          | 2.7333| 2.2263| 0.8448|
|      | St.   | Dev         | 0.5936| 0.5238| 0.2520|
| JY   | Mean  | 60          | 3.0000| 1.9842| 0.7569|
|      | St.   | Dev         | 0.7559| 0.4742| 0.2185|

* na = Observed number of alleles
* ne = Effective number of alleles (Kimura and Crow (1964))
* I = Shannon's Information index (Lewontin (1972))

| Name | Locus | Sample Size | Obs_Hom | Obs_Het | Exp_Hom* | Exp_Het* |
|------|-------|-------------|---------|---------|----------|----------|
| YJ   | Mean  | 60          | 0.3400  | 0.6600  | 0.5156   | 0.4844   |
|      | St.   | Dev         | 0.2906  | 0.2906  | 0.1453   | 0.1453   |
| YY   | Mean  | 60          | 0.2267  | 0.7733  | 0.4762   | 0.5238   |
|      | St.   | Dev         | 0.2348  | 0.2348  | 0.1119   | 0.1119   |
| JJ   | Mean  | 60          | 0.2178  | 0.7822  | 0.4601   | 0.5399   |
|      | St.   | Dev         | 0.2856  | 0.2856  | 0.1441   | 0.1441   |
| JY   | Mean  | 60          | 0.3467  | 0.6533  | 0.5278   | 0.4722   |
|      | St.   | Dev         | 0.3511  | 0.3511  | 0.1493   | 0.1493   |

* Expected homozygosty and heterozygosty were computed using Levene (1949)

3.2. Genetic Differentiation
The mean Fst values of YJ, YY, JJ and JY calculated by POPGENE 32 were respectively 0.0802, 0.0477, 0.0471, and 0.0376 (listed in Table 4). The mean Fst value indicated weak differentiation between all the samples except for the YJ populations. Genetic variation of YJ among and within populations was respectively 8.02% and 91.98%, and it indicated moderate differentiation.

The Fis value per locus indicated that, a) one loci BJH6 of YY and JJ existed heterozygote deficiency, and the other locus existed heterozygosis excess, b) two locus BJY9 and BJH13 of YJ existed heterozygote deficiency, and the other locus existed heterozygosis excess, c) three locus BJY9, BJH6 and BJH13 of JY existed heterozygote deficiency, and the other locus all existed heterozygosis excess.
**Table 4. Summary of F-Statistics and gene flow for all locus**

| Locus | Sample Size | Fst | Fit | Nm* | Locus | Sample Size | Fst | Fit | Nm* |
|-------|-------------|-----|-----|-----|-------|-------------|-----|-----|-----|
| Group 1 | YJ | | | | Group 3 | JJ | | | |
| BJY9 | 60 | 0.1304 | 0.1823 | 0.0596 | 3.9429 | BJY9 | 60 | -0.2500 | -0.0714 | 0.1429 | 1.5000 |
| BJY11 | 60 | -0.5000 | -0.4815 | 0.0123 | 20.0000 | BJY11 | 60 | -0.3978 | -0.3589 | 0.0279 | 8.7187 |
| BJY17 | 60 | -0.2060 | -0.1940 | 0.0100 | 24.8750 | BJY17 | 60 | -0.4474 | -0.3808 | 0.0460 | 5.1818 |
| BJY18 | 60 | -0.5929 | -0.4286 | 0.1032 | 2.1731 | BJY18 | 60 | -0.7143 | -0.6667 | 0.0278 | 8.7500 |
| BJH5 | 60 | -0.8182 | -0.1765 | 0.3529 | 0.4583 | BJH5 | 60 | -0.6418 | -0.5789 | 0.0383 | 6.2813 |
| BJH6 | 60 | -0.1111 | -0.0942 | 0.0152 | 16.2000 | BJH6 | 60 | 0.5833 | 0.6407 | 0.1377 | 1.5652 |
| BJH10 | 60 | -0.6822 | -0.6514 | 0.0183 | 13.3750 | BJH10 | 60 | -0.6484 | -0.6129 | 0.0215 | 11.3750 |
| BJH12 | 60 | -0.5512 | -0.4520 | 0.0640 | 3.6588 | BJH12 | 60 | -0.7241 | -0.7241 | 0.0000 | **** |
| BJH13 | 60 | 0.0741 | 0.4163 | 0.3696 | 0.4263 | BJH13 | 60 | -0.4607 | -0.2621 | 0.1359 | 1.5893 |
| BJH14 | 60 | -0.6111 | -0.5861 | 0.0155 | 15.8824 | BJH14 | 60 | -1.0000 | -1.0000 | 0.0000 | **** |
| BJH16 | 60 | -0.6997 | -0.6965 | 0.0019 | 132.3750 | BJH16 | 60 | -0.8072 | -0.7928 | 0.0080 | 31.1250 |
| BJH17 | 60 | -1.0000 | -1.0000 | 0.0000 | **** | BJH17 | 60 | -0.5152 | -0.5000 | 0.0100 | 24.7500 |
| BJH19 | 60 | -0.5432 | -0.3228 | 0.1429 | 1.5000 | BJH19 | 60 | -0.6667 | -0.5909 | 0.0455 | 5.2500 |
| BJH20 | 60 | -0.4925 | -0.4652 | 0.0183 | 13.4000 | BJH20 | 60 | -0.7647 | -0.6129 | 0.0860 | 2.6563 |
| BJY12 | 60 | -0.3816 | -0.2897 | 0.0665 | 3.5077 | BJY12 | 60 | -0.4607 | -0.3879 | 0.0498 | 4.7679 |
| Mean | 60 | -0.5065 | -0.3857 | 0.0802 | 2.8683 | Mean | 60 | -0.5728 | -0.4987 | 0.0471 | 5.0557 |
| Group 2 | YY | | | | Group 4 | JY | | | |
| BJY9 | 60 | -0.3208 | -0.2281 | 0.0702 | 3.3125 | BJY9 | 60 | 0.6226 | 0.6768 | 0.1436 | 1.4906 |
| BJY11 | 60 | -0.6129 | -0.5358 | 0.0478 | 4.9821 | BJY11 | 60 | -0.6938 | -0.6578 | 0.0213 | 11.5125 |
| BJY17 | 60 | -0.4103 | -0.3808 | 0.0209 | 11.7000 | BJY17 | 60 | -0.2000 | -0.1273 | 0.0605 | 3.8793 |
| BJY18 | 60 | -0.5789 | -0.4286 | 0.0952 | 2.3750 | BJY18 | 60 | -1.0000 | -1.0000 | 0.0000 | **** |
| BJH5 | 60 | -0.8182 | -0.6667 | 0.0833 | 2.7500 | BJH5 | 60 | -0.2000 | -0.1111 | 0.0741 | 3.1250 |
| BJH6 | 60 | 0.0625 | 0.1667 | 0.1111 | 2.0000 | BJH6 | 60 | 0.3443 | 0.3634 | 0.0292 | 8.3182 |
| BJH10 | 60 | -0.6854 | -0.6014 | 0.0498 | 4.7679 | BJH10 | 60 | -0.5635 | -0.4516 | 0.0716 | 3.2430 |
| BJH12 | 60 | -1.0000 | -1.0000 | 0.0000 | **** | BJH12 | 60 | -0.4987 | -0.4512 | 0.0317 | 7.6382 |
| BJH13 | 60 | -0.2987 | -0.1494 | 0.1149 | 1.9250 | BJH13 | 60 | 0.5062 | 0.5186 | 0.0251 | 9.7200 |
| BJH14 | 60 | -1.0000 | -1.0000 | 0.0000 | **** | BJH14 | 60 | -0.8519 | -0.7928 | 0.0319 | 7.5937 |
| BJH16 | 60 | -0.8072 | -0.7928 | 0.0080 | 31.1250 | BJH16 | 60 | -0.7073 | -0.6935 | 0.0081 | 30.7500 |
| BJH17 | 60 | -0.7808 | -0.7647 | 0.0090 | 27.3750 | BJH17 | 60 | -0.9398 | -0.9355 | 0.0022 | 112.1250 |
| BJH19 | 60 | -0.3684 | -0.3220 | 0.0339 | 7.1250 | BJH19 | 60 | -0.7891 | -0.7266 | 0.0349 | 6.9044 |
| BJH20 | 60 | -0.6000 | -0.5254 | 0.0466 | 5.1136 | BJH20 | 60 | -0.4570 | -0.3808 | 0.0523 | 4.5300 |
| BJY12 | 60 | -0.4063 | -0.3043 | 0.0725 | 3.2000 | BJY12 | 60 | -0.2000 | -0.1392 | 0.0506 | 4.6875 |
| Mean | 60 | -0.6037 | -0.5272 | 0.0477 | 4.9923 | Mean | 60 | -0.4620 | -0.4069 | 0.0376 | 6.3909 |

Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

### 3.3. Genetic Identity, Genetic Distance and UPGMA

POPGENE 32 was used to analyze the mean genetic distance and genetic identity among the four populations (listed in Table 5). The mean genetic distance between JJ and YY was the closest (0.0097), next to YY and JY (0.0655), and that between JJ and YY was the most distant (0.1096). The mean genetic identity between JJ and JJ was the highest (0.9903), next to YY and YY (0.9366), and that between JJ and YY was the lowest (0.8962).

Furthermore, distances between pairs of individuals based on allele sharing were calculated using
MEGA 4.0 of unweighted pair group method using arithmetic mean (UPGMA). The result (Figure 1) showed JY and YJ firstly clustered together, and then they clustered together with the self-bred offspring (JJ and YY). It indicated that the relationship between JY and the self-bred offspring was further than that between YJ and the self-bred offspring.

**Table 5. Genetic identity and genetic distance of the four populations**

| pop ID | YJ   | YY   | JJ   | JY   |
|--------|------|------|------|------|
| YJ     | ****| 0.9366| 0.9435| 0.9732|
| YY     | 0.0655| ****| 0.9903| 0.8962|
| JJ     | 0.0581| 0.0097| ****| 0.9202|
| JY     | 0.0272| 0.1096| 0.0832| ****|

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

**Figure 1.** Dendrogram analysis of the four populations based on Nei’s (1978) standard genetic distance.

4. Discussion

4.1. Genetic Variation

Analysis of microsatellites diversity had been reported in many hybrids of different sources and different varieties [17, 18]. For YJ, YY, JJ and JY, the range of na (observed number of alleles) of 15 locus were from 2.00 to 5.00, and the range of ne (effective number of alleles) were from 1.14 to 2.90. There was no significant difference with the mean na and ne between the intraspecific hybrids and self-bred Steelhead trout and golden rainbow trout. The above result was probable to have relations with the selected microsatellite sequences. Shannon’s information index was introduced in population genetics by Lewontin [19], and could reflect the extent of individuals’ genetic variation among the populations. In this study, the mean of I (Shannon’s Information index) (range from 0.7569 to 0.8448) were higher than 0.5, and indicated that the alleles were well-distributed and high polymorphism. Therefore, the 15 microsatellite primers were reliable to be used in analysis of genetic diversity.

4.2. Genetic Differentiation

To determine the extent of genetic variation within and between populations for this locus, F statistics (Fis, Fit, and Fst) was obtained [20, 21]. The statistic Fis estimates the variation inside populations. There was heterozygote deficiency if Fis value is positive and heterozygote excess if Fis value is negative. The mean Fis value of the four populations (-0.5065, -0.6037, -0.5728, -0.4620), showed an average excess of heterozygote proportion. But there was one loci BJH6 of YY and JJ, whose value was respectively of 0.0625, 0.5833, and it indicated that YY and JJ both existed one loci heterozygote deficiency. Two locus BJY9 and BJH13 of YJ existed heterozygote deficiency, with the Fis values respectively 0.0625, 0.5833, and heterozygote deficiency existed in three locus BJY9, BJH6 and BJH13 of YJ, with the Fis values respectively of 0.6226, 0.3443, 0.5062. Although diversity was high in the four populations, deficiency heterozygotes were detected. It is possible that this is a result of null alleles at loci BJY9, BJH6 and BJH13 or increased homozygosity of high frequency alleles. The significantly positive overall estimates of Fis in the JY and YJ populations reflected the occurrence of
low proportional levels of inbreeding in the intraspecific hybridization.

Parameter FST is the correlation between random gametes from different individuals within subpopulations with respect to the total population and is a measure of the differentiation of subpopulations. The magnitude of genetic differentiation among the stocks of S. brasiliensis, was determined by scale proposed by Wright [22], where FST values from 0 to 0.05 indicate little genetic differentiation; from 0.05 to 0.15 moderate differentiation; from 0.15 to 0.25 high differentiation; above 0.25 very high differentiation. According to the scale proposed by Wright [22], Fst values of YY, JY and JJ (0.0477, 0.0376, and 0.0471) indicated little genetic differentiation, and Fst values of YJ (0.0802) indicated moderate genetic differentiation. Therefore, hybridization of (steelhead ♀ × golden rainbow trout ♂) was not the best way to get authentic golden rainbow trout

4.3. Dendrogram and Inbreeding Depression
From the cluster diagram, we found that the two intraspecific hybrids of steelhead trout and golden rainbow trout showed a closer relationship to the golden rainbow trout. The possible reason was that more genetic material of golden rainbow trout were inherited to their hybrids, or genetic recombination, deletion, insertion, and mutations.

The two principal theories of the causal mechanism for inbreeding depression are the partial dominance hypothesis and the over dominance hypothesis [23-26]. According to the first hypothesis, inbreeding increases the frequency of homozygous combinations of deleterious recessive alleles thereby decreasing fitness, whereas the overdominance hypothesis posits that inbreeding increases homozygosity and thus reduces the frequency of the superior heterozygotes (Derek 2002). Which of these is the most important mechanism of inbreeding depression was still in dispute [27, 28]. Larger deficits in heterozygotes and higher homozygosity of YJ and JY offer supporting evidence that heterozygote deficiency and homozygosity increasing played an important role in intraspecific hybridization depression. Our result was partial in favor of dominance overdominance, and consistent with study of flatfish [29].

5. Conclusion
our data suggested that the best way to get authentic golden rainbow trout was selective breeding from a single mutated color variant of Oncorhynchus mykiss (for example, Coastal rainbow trout, Athabasca rainbow trout, Kamloops rainbow trout, Mexican rainbow trout, et al.), not intraspecific hybridization of steelhead and self-breeding of golden rainbow trout.

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