Biochemical Systematics and Ecology 57 (2014) 40–47

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Genetic diversity and variation in wild populations of dark sleeper (*Odontobutis potamophila*) in China inferred with microsatellite markers

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ARTICLE INFO

Article history:
Received 31 December 2013
Accepted 4 July 2014
Available online

Keywords:
Odontobutis potamophila
Microsatellite markers
Genetic variation

ABSTRACT

Twenty-one highly variable microsatellite loci were used to investigate the genetic diversity and variation of *Odontobutis potamophila* in China. A total of 160 samples from five wild populations (Dangtu, Sheyang, Yuyao, Minjiang and Donxishan) were genotyped. All of the 21 microsatellite loci tested in this study showed polymorphism. The number of allele per locus ranged from 5.05 to 9.90. Locus 87a of Minjiang population had a 259-bp characteristic allele. The average observed heterozygosity and expected heterozygosity ranged from 0.33 to 0.62 and from 0.40 to 0.70, respectively. The pair-wise F_{ST} tests and NJ trees of the five *O. potamophlia* populations revealed that Dangtu, Sheyang, Yuyao and Dongxishan were genetically close to one another and distinct from Minjiang. Far genetic distances were observed among populations from distant geographical areas. This result provided guide for the use of *O. potamophila* breeds and the protection of the species.

1. Introduction

The freshwater sleeper *Odontobutis potamophila* (Günther, 1861) is widely distributed in the river systems of southeast China (Iwata et al., 1985). It is an important and commercially valuable fish that mainly relies on wild resources. *O. potamophila* is listed as near threatened in IUCN (International Union for the Conservation of Nature) in 2012 (Huckstorf, 2012). However, genetic information on *O. potamophila*, such as the degree of genetic variation and population structure, is scanty. Only Yan (2010) have reported on the utility of ISSR to assess genetic variation in two wild populations, and Hou et al. (2013) have reported on the utility of mtDNA control region to assess genetic diversity in five populations. However, microsatellite systems have not been used to study wild stocks of *O. potamophila*.

Microsatellite DNA marker is an appropriate tool for genetic tagging of populations, and microsatellite analysis has been used to investigate the genetic diversity of wild population of *O. potamophila* to determine whether habitat degradation or

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http://dx.doi.org/10.1016/j.bse.2014.07.002
0305-1978/

unsuitable exploitation have reduced the genetic variation in farmed populations. Our results are useful for the management of wild *O. potamophila* populations. The conservation of the genetic diversity of these wild populations is critical and requires the identification of appropriate markers. Suitable markers are also necessary for monitoring the diversity of cultured stock and for restocking the natural river systems.

2. Materials and methods

2.1. Sample collection and genomic DNA extraction

A total of 160 samples of *O. potamophila* were collected from five wild populations in China (Table 1). The collected vouchers were deposited into the Key Laboratory of Biodiversity and Biotechnology of Jiangsu Province (Fig. 1). There were no detailed records on either the constitution or the maintenance of the wild stock. Tail fin tissue was taken from each individual and DNA was extracted using the phenol-chloroform method with minor modifications (Sambrook et al., 1989).

2.2. Amplification of microsatellite loci

Polymerase chain reaction (PCR) amplification was performed using twenty-one microsatellite markers developed by Zhu et al. (2014) (Table 2). The primer sequences, microsatellite core sequences and optimum PCR amplification conditions are listed in Table 2. PCR amplification was performed in 20-μL reaction mixture containing 0.5 U *Taq* polymerase, 2 μL 10 × reaction buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 1 pM primer each and 50–100 ng template DNA. PCR amplification was performed with the following cycling parameters: an initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 1 min, 46–57 °C for 30 s, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. Subsequently, PCR products were analyzed with an ABI 3500xl sequencer, and sizes were determined with GENEMAPPER version 4.1.

2.3. Statistical analysis

The number of alleles per locus and the range of allele size were calculated using the Arlequin software v3.5 (Excoffier and Lischer, 2010). Arlequin was also used to estimate Hardy–Weinberg equilibrium (*HWE*), Markov-chain method (dememorization = 10,000, batch = 1000, iterations per batch = 1000), linkage disequilibrium (LD), population pair-wise F_{ST} (Slatkin, 1985) and F_{ST} significance values across population (Weir and Cockerham, 1984). Sequential Bonferroni adjustments (Rice, 1989) were applied to correct the effect of multiple tests. Observed heterozygosity (H_O) and expected heterozygosity (H_E) for each sample and population were calculated using POPGENE version 1.32 (Yeh et al., 1999). After the construction of a genetic distance matrix based on a set of gene frequencies in different populations (Nei, 1972), a neighbor-joining tree was constructed for each replicated genetic distance matrix with bootstrapping of 1000 replications using NEIGHBOR in PHYLIP 3.5 (Felsenstein, 1993).

Inbreeding coefficient values (F_{IS}) were calculated using the Genepop 4.2 package (Raymond and Rousset, 2003). The Markov chain was set to 1 million iterations with 100,000 steps for burn-in. The presence of null alleles was tested using MICROCHECKER (Van Oosterhout et al., 2004), and then the genotype frequency of populations with null alleles were adjusted accordingly.

Usually, population determination is based on geographical origin of samples. However, general graphical methods (UPGMA or neighbor joining) are only loosely connected to statistical procedures, allowing the identification of homogeneous clusters of individuals. Therefore, the Bayesian clustering approach implemented in STRUCTURE version 2.2 (Pritchard et al., 2000) was used to determine whether it was consistent with the genetic information. The number of clusters (K) was determined using the admixture ancestry model and the correlated allele frequencies were determined by testing $K = 1$ to 10 with 10 runs at each K for the five populations. We performed these analyses with a 'burn in' period setting of 10^5 and 10^6 Monte-Carlo Markov Chain (MCMC) repetitions in order to estimate the number of populations (K). The most appropriate K

Table 1
Information of the sampling sites.

Populations (abbreviation)	Latitude longitude	Sampling date	Sample number
Dangtu (DT)	N31°25′37″ E118°42′59″	Nov. 2011	32
Sheyang (SY)	N33°18′31.03″ E119°37′36.98″	Jan. 2012	32
Yuyao (YY)	N30°0′16.49″ E121°3′6.33″	Jan. 2011	32
Minjiang (MJ)	N25°50′55.64″ E118°57′56.31″	Apr. 2012	32
Dongxishan (DXS)	N30°59′58.16″ E120°27′21.02″	Dec. 2011	32