



Genetic differentiation of *Octopus minor* (Mollusca, Cephalopoda) off the northern coast of China as revealed by amplified fragment length polymorphisms

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ABSTRACT. *Octopus minor* (Sasaki, 1920) is an economically important cephalopod that is found in the northern coastal waters of China. In this study, we investigated genetic differentiation in fishery populations using amplified fragment length polymorphisms (AFLPs). A total of 150 individuals were collected from five locations: Dalian (DL), Yantai (YT), Qingdao (QD), Lianyungang (LY), and Zhoushan (ZS), and 243 reproducible bands were amplified using five AFLP primer combinations. The percentage of polymorphic bands ranged from 53.33 to 76.08%. Nei's genetic identity ranged from 0.9139 to 0.9713, and the genetic distance ranged from 0.0291 to 0.0900. A phylogenetic tree was

constructed using the unweighted pair group method with arithmetic mean, based on the genetic distance. The DL and YT populations originated from one clade, while the QD, LY, and ZS populations originated from another. The results indicate that the *O. minor* stock consisted of two genetic populations with an overall significantly analogous F_{ST} value (0.1088, $P < 0.05$). Most of the variance was within populations. These findings will be important for more sustainable octopus fisheries, so that this marine resource can be conserved for its long-term utilization.

Key words: *Octopus minor*; Population structure; North China coast; AFLP

INTRODUCTION

Cephalopods, commonly known as the octopuses, squids, and cuttlefishes, together with the vampire squid and *Nautilus*, are a well-defined class of Mollusca (Boyle and Rodhouse, 2005). There are about 700 described species of living cephalopods and around 17,000 fossil species (Brusca and Brusca, 1990). Octopuses are widespread in the world's oceans, from the poles to the tropics, and the genus *Octopus* accounts for one-third of the total number of cephalopod species (Huffard, 2007).

Octopus minor (Sasaki, 1920) is a benthic, littoral species that lives in the subtidal zone down to a depth of 150 m, and is widely distributed in the coastal waters of China, the Korean Peninsula, as well as the Western Pacific waters of the Japanese archipelago (Okutani et al., 1987; Lu et al., 2012). *Octopus variabilis* has been used as a synonym for the species by some researchers (e.g., Yamamoto, 1942; Dong, 1988; Lu et al., 2012). It is an economically important cephalopod in northern China, because not only does it have a high protein content but also considerable medicinal value (Qian et al., 2010).

With exports to Korea and Japan, the fishing pressure on this species is increasing. To conserve it, it is imperative to understand its population structure and identify fishing limits, based on genetic data (Thorpe et al., 2000; Kang et al., 2012). Recently, the increased use of molecular markers, such as amplified fragment length polymorphisms (AFLPs), has revealed the genetic structures of marine populations (Luo et al., 2010). The importance of the genetic characterization of populations has led to the development of the "genetic stock concept" (Carvalho and Hauser, 1994).

An effective management program for the sustainable use and conservation of *O. minor* is necessary. Such a program requires knowledge of the present genetic status of the species, and the ability to monitor genetic changes caused by overharvesting. Its population biology is poorly documented (Gao et al., 2009); therefore, the objective of the present study was to use highly variable AFLP markers to evaluate the level of genetic variability and genetic structuring of *O. minor* off the northern coast of China.

MATERIAL AND METHODS

Sample collection

Samples were collected from five localities in the coastal waters of China: Dalian (DL,

38.93°N, 122.02°E), Yantai (YT, 37.67°N, 121.17°E), Qingdao (QD, 35.82°N, 120.37°E), Lianyungang (LY, 34.75°N, 119.83°E), and Zhoushan (ZS, 30.25°N, 122.64°E) from December 2007 to March 2008 (Table 1 and Figure 1). Thirty individuals were randomly selected from each population, and muscle tissue was cut from the mantle of each individual and stored in 1.5-mL centrifuge tubes at -80°C.

Table 1. Number of samples, date, and average body weight (g) of five geographic populations *O. minor* in the coastal waters of China.

Populations	Abbreviation	Number	Date	Average body weight (\pm SD)
Dalian	DL	30	2008.01.22	254.65 \pm 48.83
Yantai	YT	30	2008.01.23	263.11 \pm 36.41
Qingdao	QD	30	2008.03.26	257.42 \pm 58.74
Lianyungang	LY	30	2007.12.23	195.76 \pm 65.43
Zhoushan	ZS	30	2008.01.06	136.24 \pm 38.07



Figure 1. Sampling locations of *Octopus minor* along the coastal waters of China.

DNA extraction and AFLP analysis

Genomic DNA was extracted from 150 individuals from the five populations according to Sambrook et al. (1989) method. The extracted DNA was quantified by fluorimetry, and was then diluted to 25 ng/ μ L. The AFLP analysis followed the modified protocol as described by Vos et al. (1995). The adapter and primer sequences are presented in Table 2. To check the reproducibility of the results and ensure the comparison of similar bands on the two gels required for the analysis of each primer combination, a sample of control DNA was included on the gels. In addition, several randomly selected accessions were reprocessed and run on separate gels.

Table 2. Adapter and primer sequences used in AFLP analysis (Vos et al., 1995).

Primer/Adapter	Sequence (5'-3')
<i>Eco</i> RI-adapter	5'-CTCGTAGACTGCGTACC-3' 3'-CTGACGCATGGTTAA-5'
<i>Mse</i> I-adapter	5'-GACGATGAGTCCTGAG-3' 3'-ACTCAGGACTCAT-5'
<i>Eco</i> RI-adapter	GACTGCGTACCAATTC-
<i>Mse</i> I-adapter	GATGAGTCCTGAGTAA-

Data analysis

For each gel generated from each primer pair, the numbers of observable and monomorphic AFLP bands were counted. Clear polymorphic AFLP bands were scored as 1 (present) or 0 (absent). The bands were first analyzed for AFLPs by counting the number of polymorphic bands and calculating their frequencies with respect to the primer pairs, samples, and populations. To compare AFLPs between the populations, the numbers of polymorphic bands were plotted against their frequencies of occurrence in each population.

Calculations of heterogeneity or total genetic diversity were conducted according to Nei and Li (1979). For this estimation, each band was considered as a biallelic locus, so that if a band was present it was one allele, and if it was absent it was the alternative allele. The parameters *P* (percentage of polymorphic bands), *H* (Nei's gene diversity), and *I* (Shannon's Information index) were separately analyzed using POPGENE 1.31 (Yeh et al., 1999). The five geographical populations were clustered using the unweighted pair group method with arithmetic mean (UPGMA) in MEGA 4.1 (Tamura et al., 2007).

Analysis of molecular variance (AMOVA; Excoffier et al., 1992) of AFLP variation between populations was performed using Arlequin 3.0 (Excoffier et al., 2005). The value of F_{ST} was also ascertained.

RESULTS

AFLP variation

A total of 243 AFLP bands were amplified from the five AFLP primer pairs (Table 3). The number of observable bands per primer pair ranged from 42 to 60, with an average of 48.6. *P* ranged from 53.33 to 76.08%. No AFLP band was found to be specific to only one population, and the number of polymorphic bands differed between primer pairs. The number of AFLP bands obtained from the primer pair E-ATG/M-CCT was the highest, and the bands from the primer pair E-ACA+M-CAT had the highest *P* (76.08%). There were 155 polymorphic bands among the 243 AFLP bands, and *P* was 63.79%.

AFLP diversity between populations

A comparison of AFLP band genetic diversity in the five populations was performed (Table 4). The QD population had the highest *H* value ($H = 0.3075$), and the YT population has the lowest ($H = 0.2374$). *I* values exhibited the same pattern (QD, $I = 0.4552$; YT, $I = 0.3528$). The results demonstrate that all of the populations had a high genetic diversity, but the QD

population was more diverse than the others.

Table 3. Number of bands generated by primer combinations.

Primer pair	No. of AFLP bands	No. of polymorphic bands	Percentage of polymorphic bands
E-AAC/M-CTT	42	28	66.67
E-ACA/M-CAT	46	35	76.08
E-ATG/M-CCT	60	32	53.33
E-ATG/M-CTG	42	26	61.90
E-ACT/M-CAC	53	34	64.15
Total	243	155	
Average	48.6	31	63.79

Table 4. Genetic diversity of five geographical populations of *Octopus minor*.

Population	Observed No. of alleles (N_A)	Nei's gene diversity (H)	Shannon's Information index (I)
DL	1.6451	0.2476	0.3671
YT	1.6203	0.2374	0.3528
QD	1.8057	0.3075	0.4552
LY	1.6667	0.2681	0.3926
ZS	1.6498	0.2417	0.3599

Among the five geographically different populations, Nei's genetic identity (Table 5) ranged from 0.9139 to 0.9713, and the genetic distance ranged from 0.0291 to 0.0900. The genetic distance between QD and LY was the shortest (0.0291), and between DL and ZS was the longest (0.0900). The highest Nei's genetic identity was between QD and LY (0.9713), and the lowest was between DL and ZS (0.9139), indicating that differentiation between QD and LY was lower than that between the other populations.

Table 5. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) of *Octopus minor*.

	DL	YT	QD	LY	ZS
DL	-	0.9463	0.9184	0.9234	0.9139
YT	0.0552	-	0.9256	0.9364	0.9299
QD	0.0852	0.0773	-	0.9713	0.9262
LY	0.0797	0.0657	0.0291	-	0.9617
ZS	0.0900	0.0727	0.0767	0.0391	-

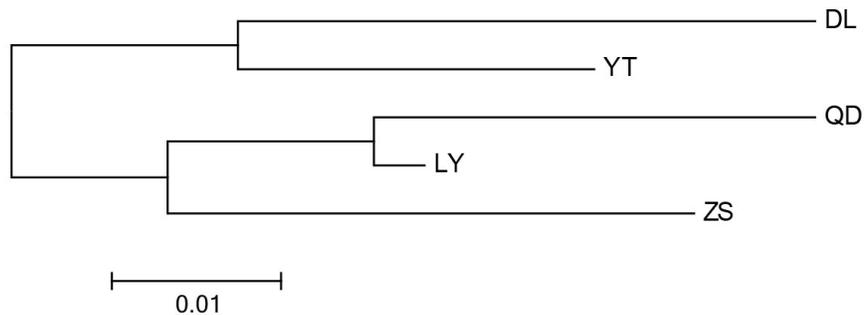
Based on the F_{ST} estimation from the AMOVA (Table 6), 10.88% of the variation was between populations and 89.12% was within populations. Therefore, the diversity between populations was much lower than within populations.

Based on the AFLP data, an UPGMA tree was constructed based on the five populations (Figure 2). The five populations could be classified into two clades: the DL and YT populations may have originated from one clade, while the QD, LY, and ZS populations originated from another.

Table 6. Analysis of molecular variance (AMOVA) among 5 populations of *Octopus minor*.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variance
Among populations	4	494.067	3.15749 ^{va}	10.88
Within populations	145	4174.833	28.79195 ^{vb}	89.12
Total variation	149	4668.900	31.94944	

$F_{ST} = 0.1088$ ($P < 0.05$).

**Figure 2.** UPGMA tree of the five geographic populations in *Octopus minor*.

DISCUSSION

Genetic diversity of *O. minor*

To our knowledge, this is the first study that has attempted to assess the genetic diversity and population structure of *O. minor* using AFLP markers. Five highly polymorphic and reproducible primer pairs were selected. The overall percentage of polymorphic loci in the five populations was 63.79%, which is much higher than the data obtained for allozyme loci (20-30%) (Gao et al., 2009) and mitochondrial cytochrome *c* oxidase subunit I sequences (Sun et al., 2010), but is similar to that found for *Octopus ocellatus* (62.03 to 67.93%) that was analyzed by six AFLP primer combinations (Zhang et al., 2009). This result demonstrates that AFLP obtained more information than the other two methods of genetic diversity analysis.

Intraspecific population differentiation and limited gene flow of *O. minor*

In the present study, the genetic distance between the five geographical groups ranged from 0.0291 to 0.0900, which is greater than the data obtained from COI sequences (0.0029-0.0077) (Sun et al., 2010). However, the topologies of the neighbor-joining and UPGMA phylogenetic trees that were based on the COI sequences were similar to the tree constructed by AFLP, and both approaches indicated that the five populations were clustered into two major clades. The DL and YT populations may have originated from one clade, while the QD, LY, and ZS populations originated from another. Chang et al. (2010) analyzed seven geographical populations of this species based on COI sequences, and placed DL, YT, QD, LY, and ZS into the same group.

On the basis of the results of the present study, *O. minor* stock should be regarded as two different genetic populations ($F_{ST} = 0.1088$). The reasons for this may be related to the

ecological environment, geographical conditions, or *O. minor* breeding behavior. Shandong Peninsula may be a natural barrier to gene exchange. In addition, bottom-dwelling in shallow coastal waters and troglodytism could also result in the limited migration of *O. minor*. Therefore, gene flow is difficult between individuals in the Yellow Sea and the Bohai Sea (Palumbi, 2003). From a management perspective, these genetically isolated populations should be treated as separate management units. Stock enhancement to ameliorate the effects of overexploitation of natural *O. minor* populations should be conducted using local populations as founders.

Zuo et al. (2011) used 12 microsatellite DNA markers to estimate the *O. minor* population at Rongcheng (37.16°N, 122.41°E) (Figure 1), which is located between YT and QD. Further studies will provide more information about the two genetic populations (Gao, 2014).

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