

## GENETIC DIVERSITY IN POPULATIONS OF *SEPIELLA JAPONICA* BASED ON THE MITOCHONDRIAL DNA SEQUENCE ANALYSIS

Xiaodong Zheng\*, Qi Li, Zhaoping Wang, Ruihai Yu,  
Chuanyuan Tian and Rucai Wang

*The Key Lab of Mariculture Certificated by the Ministry of Education  
Ocean University of China, Qingdao 266003, China*

\*Corresponding author: Tel. +86-532-2032873

E-Mail: xdzheng@ouc.edu.cn

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**ABSTRACT:** Part of the 16S rRNA gene was amplified with PCR and sequenced for 57 individuals from 5 populations of common Chinese cuttlefish *Sepiella japonica*: three from the South China Sea, one from East China Sea and one from Nagasaki water (Japan). The result showed that a total of 5 nucleotide positions were found to have insertions/deletions among these individuals, and 13 positions were examined to be variable in all the sequences, which ranged from 494 to 509 base pairs. All of the individuals were grouped into 7 haplotypes (h1–h7). The individuals from Nagasaki belonged to h1 and the h3 haplotype was found only in the coastal waters of China. A↔G transition in Nucleotide 255 was suggested to be taken as a kind of genetic marker to identify the populations distributed in East-South China Sea and the Nagasaki water of Japan.

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### INTRODUCTION

Cephalopods are a potentially valuable protein resource and highly promising animals for mariculture because of such characters as high nutritional qualities, short life span and extremely rapid growth (Nesis, 1987). China is one of the major countries of cephalopod fisheries. It boasts rich species resources: 101 species belonging to 6 orders, 21 families and 45 genera (Huang, 1994) have been discovered.

*Sepiella japonica* Sasaki, 1929 is an important and valuable fishery resource in China. Its output has been up to 60% of the total amount of cephalopods caught in the 1980s. A great deal of fundamental and implicational research about the species has been carried out in China, with emphasis on the following subjects: fauna systematics, morphology, anatomy, embryology and development, ecology, physiology, as well as fishery resources since 1930s (Wu and Tang, 1990; Dong, 1993; Zhang, *et al.*, 1997). However, aspects of its genetics, such as population biology and molecular genetics, are generally poorly documented.

Intraspecific sequence variation within mitochondrial DNA (mtDNA) has proven to be a powerful tool for studying population structure in marine organism (Carvalho and Pitcher, 1995). To date, few population geneticists have made use of DNA sequences of population samples and the corresponding statistical tools for shellfish research. The authors (Zheng, *et al.*, 2001) discussed the genetic diversity of 5 populations of *S. japonica* using allozyme electrophoresis and cytochrome oxidase subunit I (COI) gene sequence analysis. But this study showed very low levels of the polymorphism.

Boulding *et al* (1993) illustrated high frequency of genetic variation in a bred population and two wild populations of the scallop, *Patinopecten yessoensis* using the 16S rRNA gene sequence analysis. In the present paper we further investigated genetic diversity of some selected samples of the cuttlefish, *Sepiella japonica*, from both China and Japan by examining nucleotide sequence of part of 16S rRNA gene in the mtDNA to research its population structure for providing useful information for resource management.

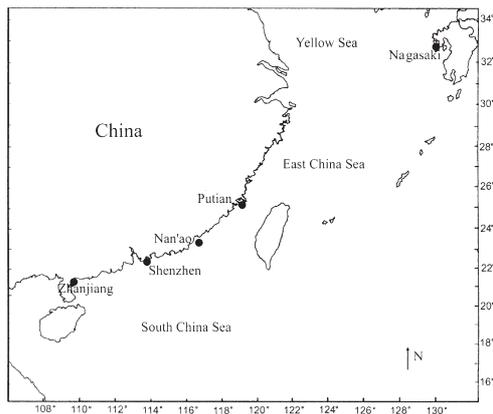
## MATERIALS AND METHODS

### Samples

The fresh common Chinese cuttlefish *S. japonica* samples were collected from Nagasaki (Japan) to Zhanjiang in the waters of China (Fig. 1). 57 individuals from 5 locations were used: three from the South China Sea (Nan'ao, Shenzhen and Zhanjiang), one from East China Sea (Putian) and one from Japan (Nagasaki). Fresh muscle tissues were removed for DNA analysis.

### DNA extraction, Amplification and Sequencing

Total genomic DNA was extracted using a CTAB method modified from Winnepenninckx *et al.* (1993). Region of the 16S rRNA gene was PCR amplified. The primers described by Anderson (2000) in use for the amplification were: D16SAR 5'-CGC CTG TTT AHY AAAA ACAT-3', D16SBR 5'-CCG GTC TGA ACT CAG MTC AYG T-3'. The temperature regime for 35 cycles was 1 min at 94°C, 1 min at 51°C, and 1 min at 72°C. A total volume of 25 µL reactions consists of 0.5 units of *Taq* (TaKaRa), 0.5 µM each primer, 0.2 µM each dNTP, 2.5 µL of 10×buffer and 4 µL (30–50 ng) of template DNA. Purification and sequencing were the same as the processing used previously by the authors (Zheng, *et al.*, 2001).



**Figure 1.** Location of *Sepiella japonica* collected in the coastal waters of China and Nagasaki port in Japan.

### Sequence Difference Analyses

All of the sequences were aligned with the software CLUSTAL W (Thompson, *et al.*, 1994.). Bootstrap analysis with 1000 replicates was performed by the SEQBOOT and CONSENSE programs from the PHYLIP package (Felsenstein, 1995). Pairwise sequence divergences among the 5 samples were estimated by the DNADIST program from PHYLIP according to Kimura's two-parameter model (Kimura, 1980).

## RESULTS

### Sequence Variation of the Partial 16S rRNA Gene

Partial segment of the 16S rRNA gene, which ranged from 494 to 509 base pairs, was sequenced for 57 individuals from the 5 locations (GenBank Accession nos. AF369118-9, AF369958-60). The percentage of A+T (72.79%) of the individuals from Nagasaki was the lowest in all the *S. japonica* samples. A total of 5 nucleotide positions (position 50, 250, 257, 258, and 267) were found to have insertions/deletions (Fig. 2) among these individuals. Meanwhile 13 nucleotides (No. 14, 15, 198, 246, 255, 259, 309, 330, 360, 372, 414, 471, 482) were examined to be variable, including 9 transitions (A/G or T/C) and 4 transversions (A/T).

### Analysis of Relationships and Occurrence Frequency of Haplotypes

7 haplotypes (h1–h7) were found in 57 individuals from 5 samples (Table 1). Most of the individuals were grouped into two kinds of haplotypes (h1 and h3), which differed only in one purine transition (G↔A). The h3 haplotype was the most common in the Putian, Nan'ao, Shenzhen and Zhanjiang samples, the pooled frequency of which was about 58% (33/57). All the sequences in the Nagasaki sample were the same, belonging to h1. The h4 was the third most common haplotype, following h1. The other haplotypes were found respectively in 1 or 2 individuals. There are two kinds of haplotypes (h6 and h7) only found in the most southerly site (Zhanjiang sample). Genetic distance was close to zero within the individuals by the DNADIST program from PHYLIP according to Kimura's two-parameter model.

Genetic diversity in populations of *Sepiella japonica***Table 1.** Variable nucleotide positions in part of the 16S rRNA gene region of 7 haplotypes, and number of individual of each haplotype found in each locality (Genbank Accession nos. AF369118-9, AF369958-60)

| Haplotype | Nucleotide no. |    |    |     |     |     |     |     |     |     |     |     |     |     |     |     |     | Number of individuals in each locality |    |    |    |    |    |       |
|-----------|----------------|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|----|----|----|----|----|-------|
|           | 14             | 15 | 50 | 198 | 246 | 250 | 255 | 257 | 258 | 259 | 267 | 309 | 330 | 360 | 372 | 414 | 471 | 482                                    | NG | PT | NA | SZ | ZJ | Total |
| H1        | G              | A  | A  | G   | G   | T   | G   | —   | —   | T   | T   | G   | A   | T   | A   | C   | C   | A                                      | 9  | 1  | 1  | 1  | 1  | 13    |
| H2        | .              | .  | —  | .   | .   | .   | .   | —   | —   | .   | .   | .   | .   | .   | .   | .   | .   | .                                      | 0  | 0  | 1  | 0  | 0  | 1     |
| H3        | .              | .  | A  | .   | .   | A   | —   | —   | —   | .   | .   | .   | .   | .   | .   | .   | .   | .                                      | 0  | 10 | 7  | 8  | 8  | 33    |
| H4        | .              | .  | A  | .   | .   | A   | —   | —   | —   | .   | .   | .   | .   | .   | .   | T   | .   | .                                      | 0  | 0  | 4  | 0  | 0  | 4     |
| H5        | .              | .  | A  | .   | .   | A   | A   | T   | .   | .   | .   | .   | .   | .   | .   | .   | .   | .                                      | 0  | 1  | 1  | 1  | 0  | 3     |
| H6        | A              | .  | —  | A   | A   | —   | A   | A   | —   | A   | —   | A   | T   | A   | .   | .   | T   | T                                      | 0  | 0  | 0  | 0  | 2  | 2     |
| H7        | A              | G  | —  | A   | A   | —   | A   | A   | —   | A   | —   | A   | T   | A   | G   | .   | T   | T                                      | 0  | 0  | 0  | 0  | 1  | 1     |

Note: dashes (—) in the table indicate gaps; Dots (.) indicate identities. (See also Fig.2)

NG: Nagasaki sample; PT: Putian sample; NA: Nan'ao sample; SZ: Shenzhen sample; ZJ: Zhanjiang sample.



*Genetic diversity in populations of Sepiella japonica*

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          430      440      450      460      470      480      490
      .....|.....|.....|.....|.....|.....|.....|.....|
H1  CAAAAAATAA GATGCGACC TCGATGTTGG ATTAAAGTAA CCTTAAGGTG CAGTAGCTTT AATGGTAAAT
H2  .....
H3  .....
H4  .....
H5  .....
H6  ..... T..... .T.....
H7  ..... T..... .T.....

          500      510
      .....|.....|.....|.....|.....|.....|.....|.....|
H1  CTGTTTCGATT TTTAAACTT T
H2  .....
H3  .....
H4  .....
H5  .....
H6  .....
H7  .....

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**Figure 2.** Seven haplotypes of 16S rRNA gene sequences of *S. japonica*.

### DISCUSSION

All the 7 haplotypes were found in the 4 samples from the Chinese coastal waters (Putian, Nan'ao, Shenzhen and Zhanjiang), among which as many as 4–5 haplotypes were in the specimens of Zhanjiang and Nan'ao. However, one sample (Nagasaki, Japan) was fixed (100%) for haplotypes h1. It showed that the haplotype frequency divergence in the *S. japonica* living in the East-South China Sea was slightly higher than those from the Nagasaki water of Japan, though there were little sequence divergences such as single nucleotide difference between h1 and h3 (see Table 1). It also indicated that the Japanese and Chinese populations may be genetically distinct, and isolated from one another. Nucleotides 255 of all the sequences were mutated from adenine (A) in the specimens from the coastal waters of China to guanine (G) in the Nagasaki sample, with the exception of h2 (single individual). It is suggested that A↔G transition in the site (see figure 2) may be a kind of genetic marker to identify the populations distributed in East-South China Sea and the Nagasaki water of Japan.

As for the Chinese populations, Haplotype h3 was the most common, with percentage ranged between 50% (Nan'ao sample) and 83% (Putian sample). Haplotypes h6 and h7 differ at 14 and 16

positions (including gaps) respectively from the h3 sequence, which was around 3% sequence divergence, whereas the other haplotypes sequences were similar (difference at only one or two positions from the h3). It was possible that two highly divergent clades existed within the Chinese populations. On the other hand, these individuals may be a cryptic species, due to the fact that h6 and h7 were only found in the Zhanjiang sample located in the most southerly site of China.

Pelagic organisms in the open ocean are generally regarded as having low levels of population differentiation, resulting from probable ample opportunities of dispersal in various life history stages and the lack of physical barriers in the environment. In recent years, some species, which were large pelagic animals with considerable mobility, have been shown with virtually none or only very low levels of genetic diversity, *e.g.* diamond-shaped squid *Thysanoteuthis rhombus* (Kitaura, *et al.*, 1998), swordfish (Rosel and Block, 1996), *Illex argentinus* (Carvalho, *et al.*, 1992). The result in this study, together with the previous research of allozymic and COI gene analysis (Zheng, *et al.*, 2001), suggested that the cuttlefish *S. japonica*, as a large pelagic animal, also possessed homogeneous population structure similar to that observed in those large mobile fishes and squids.

In brief, we revealed low levels of genetic variation in the populations of *S. japonica* using such molecular techniques as allozymic electrophoresis, COI gene and 16S rRNA gene sequence analysis (polymorphic loci=0.226<sub>0.99</sub>, mean observed heterozygosities per locus =0.038, and  $D_{Nei} = 0.0001-0.0018$ , based on the data of allozymes (Zheng, *et al.*, 2001); Little sequence divergence among the 5 populations based on the data of COI gene (Zheng, *et al.*, 2001) and 16S rRNA gene in the paper). The application of microsatellite DNA markers, including successes in organisms with low variability detectable by other method (Hughes and Queller, 1993), offers a way forward. It has been successful in some cephalopods, such as *Illex argentinus* (Adcock, *et al.*, 1999), *Sepia officinalis* (Shaw and Perez-Losada, 2000), *Octopus vulgaris* (Greatorex, *et al.*, 2000).

We are trying to use this technique and more other DNA markers to further examine the genetic diversity of *S. japonica*.

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