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P15

Low Genetic Diversity of Oval Squid *Sepioteuthis* cf. *lessoniana* 2 in Japan Inferred from Non-coding Region of Mitochondrial DNA.

Misuzu Aoki[1)], Hideyuki Imai[2)], Tohru Naruse[3)], Yuzuru Ikeda[2)]

[1]Graduate School of Engineering and Sciences, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

[2]Faculty of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

[3]Faculty of Science, National University of Singapore, 14 Science Drive 4, Singapore 117543, Republic of Singapore

Marine organisms generally show low levels of genetic differentiation over large geographic distance. Allozyme analysis is a powerful tool for examining population structure, however complicated method and needs fresh sample. Intraspecific genetic variation within mitochondrial DNA (mtDNA) has also proven a powerful tool for examining population structure and needs only fixed alcohol samples.

The oval squid *Sepioteuthis lessoniana* is widely distributed in Indo-West Pacific area and has been regarded as a valuable fishery resource in this area. To provide useful information for resource management and protection, it is important to assess the gene flow and the genetic diversity among populations. The previous genetic studies on *S. lessoniana* have provided two exclusive results. One group suggested that so-called "*S. lessoniana*" contains at least three biological species in the Japanese waters (*S.* cf. *lessoniana* 1-3). Furthermore, allozyme analysis on *S.* cf. *lessoniana* 2, a relatively coastal waters species, indicated that the genetic structure of the species significantly differs between populations of East China Sea and Pacific Ocean. Another group analyzed the allozyme composition of *S. lessoniana* from Japan and Thailand and indicated that oval squid has huge gene pool, across about 2,000 km.

The present study aims to assess degree of the gene flow and the genetic diversity of *S.* cf. *lessoniana* 2 from East Asia by using non-coding region of mtDNA and reappraise two exclusive previous works.

A total of 202 adult individuals including five populations in Japan, one population in Taiwan and one population in Vietnam were analyzed. Total DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform method. The original primers, SL-Ala and SL-Trp, which target a portion of transfer RNA (tRNA)-Ala and tRNA-Trp were used in polymerase chain reaction (PCR) to amplify the non-coding region 2. PCR products were purified with PCR Product Pre-sequencing Kit (usb). The nucleotide sequences were determined using CEQ 8800 (Beckman) and ABI 3700 genetic analyzers. All sequences were initially aligned using ClustalX ver.1.83.1, followed by manual editing using Mac Clade4 ver.4.08. Population genetic statistics were estimated using program ARLEQUIN ver.2.000. An analysis of molecular variation (AMOVA) was used to

test population structure within species.

The non-coding region 2 (403bp) sequences were obtained for 202 individuals from seven populations. In a total of 28 haplotypes were identified, eight haplotypes were shared among populations, the remaining twenty haplotypes were specific to one population. One haplotype (haplotype #1) accounted for 69.3% of the individual assayed. Major haplotypes in Japan were different from Taiwan and Vietnam. Haplotype #1 was a major haplotype in Japan, however a major haplotype of Taiwan and Vietnam was haplotype #2.

Five populations in Japan showed low genetic variability. Especially, genetic variability in Tokushima population was very low ($h = 0.0667$, $\pi = 0.0003$) and followed by Okinawa Island ($h = 0.1908$, $\pi = 0.0005$). On the other hand, the genetic variability in Taiwan ($h = 0.8972$, $\pi = 0.0124$) and Vietnam ($h = 0.6828$, $\pi = 0.0077$) was higher than that of Japanese populations (average; $h = 0.2583$, $\pi = 0.0024$).

The genetic structures of the seven populations were assessed by AMOVA. Genetic variation was 64.48% found among populations, whereas the variation within populations was 35.52% ($P<0.01$). The estimated Fst values for the 21 pairs of seven populations ranged from zero to 0.8381. The 11 pairs formed by combinations of Taiwan and Vietnam had significant Fst values ($P<0.01$). In contrast, values for pairs within Japan were generally low and all combinations were not significant. The exact test indicated similar results to those of Fst. However, Tokushima population was significantly different from two populations in Japan.

The results of this study indicated that Japanese populations of *S.* cf. *lessoniana* 2 have very low genetic diversity when compared with those of Taiwan and Vietnam, and Japanese populations appear to be a wide gene pool in this area. The average haplotype diversity value ($h = 0.2583$) among populations in this species was lower than that of spear squid, *Loligo bleekeri* ($h = 0.670$) around Japan. It has been known that *L. bleekeri* has seasonal population structure, which is not clear in *S. lessoniana*, *L. bleekeri* also possesses stronger swimming ability than that of *S. lessoniana*. These biological differences may have caused such a large difference in their genetic diversities for these Japanese species. In other words, low genetic diversity indicates that the effective population size of oval squid is small.

Taiwanese and Vietnamese populations of *S.* cf. *lessoniana* 2 show much higher genetic diversities. In contrast, Ishigaki population has lower genetic diversities than that of geographically closest Taiwanese population. Moreover, our result suggested that there have been only limited gene flows between Ishigaki and Taiwan population. These results suggested the presence of some barriers to prevent their gene flow. Kuroshio, a prominent current in this area, which moves at a rate nearly 50 million m^3/s, passes may have prevented the dispersal from Taiwan to Ishigaki, as the main flow of the current does not pass through around Ishigaki Island. In addition, *S.* cf. *lessoniana* 2 tends to prefer coastal water, and this habitat may cause high genetic diversities in much continuous habitat such as Taiwan and Vietnam. These environmental as well as biological factors may have formed isolated

Japanese populations with low genetic variability.