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Allozyme determination of genetic diversity in Japanese and Thai populations of Oval Squid (*Sepioteuthis lessoniana* Lesson, 1830)

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Abstract : Genetic variation and genotypic population structure of two geographic populations of oval squid(*Sepioteuthis lessoniana* Lesson, 1830), captured from Nagasaki(Japan) and Rayong(Thailand) were investigated using multi-locus allozyme analysis. On the basis of 22 loci screened, the percentage of polymorphic loci and observed heterozygosity were 45.45 % and 0.28 for Japanese and 50.00 % and 0.23 for Thai populations, respectively. These results suggest relatively high genetic diversity within the representative populations of *S. lessoniana*. At the same time, low genetic distances between these population were observed(D=0.003). A lack of genetic differentiation between the Japanese and Thai populations of *S. lessoniana* suggests a panmictic gene pool of *S. lessoniana* over a wide geographic area.

Key words : *Thailand, population genetics, squid, Sepioteuthis, electrophoresis*

1. Introduction

At least 31 cephalopod species belonging to 17 genera and 10 families are recognized in Thai waters (CHOTIYAPUTTA, 1993). The annual catch of cephalopods in Thailand accounts for 5.6 % of the total catch of all marine organisms (Department of Fisheries, 2000). Among the described species, the oval squid (*Sepioteuthis lessoniana*) is one of the most landed. This species also has potential to be cultured commerically (LEE *et al.*, 2001) and is therefore, regarded as one of the most important cephalopod species in Thailand (NABHITABHATA, 1978 and 1985).

Generally, *S. lessoniana* can be found from the sea surface down to approximately 100m in depth(ROPER, *et al.*, 1984). It is distributed over a wide geographic area covering most of the Indo-Pacific region, the Indian ocean, Thailand, Indonesia, northern Australia, north-to-central Japan and eastward of the Hawaiian Islands (CARPENTER and NIEM, 1998).

Knowledge on genetic variation levels and population differentiation of *S. lessoniana* is important for the design and implementation of appropriate fisheries management programs. However, relatively little is known about the intraspecific genetic variability of this squid in Thailand. This information is crucial to a rational decision whether the exploited species need to be conserved of further exploited (CAVALHO and HAUSER, 1994).

Accordingly, gene flow is one of the most important factors responsible for intraspecific differentiation of organisms (AVISE, 1994). WARD and GREWE (1994) compared results from population genetic studies of more than 300 animal species and concluded that mobility is the most important factor reflecting the apparent magnitude of intraspecific subdivisions. Therefore, it is not surprising that vagile organisms (high degree of gene flow) have lower levels of population differentiation than do relatively sedentary species. The appropriate genetic markers can be utilized to assist taxonomic iden-

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tification and to increase fisheries management efficiency in various taxa (KLINBUNGA *et al.*, 2000). In cephalopods, allozyme analysis has been used for population genetic and systematic studies in several loliginid squids (ALLY and KECK, 1978; CHRISTOFFERSON *et al.*, 1978; SMITH *et al.*, 1981; GARTHWAITE *et al.*, 1986; NATSUKARI *et al.*, 1986; AUGUSTYN and GRANT, 1988; CARVALHO and LONEY, 1989).

Population genetic studies in Thai squids are quite limited compared to literature available from other regions of the Western Pacific (IZUKA *et al.*, 1994, 1996; YOKOGAWA and UETA, 2000). Furthermore, there have been few publications documenting genetic divesity of *S. lessoniana* from Thai populations (see IZUKA *et al.* 1996). The objectives of this study were twofold. The first objective was to examine the level of genetic diversity of *S. lessoniana* captured from a major Thai fishing ground. The second objective, was to determine whether intraspecific population subdivision exists in representative geographic samples, by allozyme analysis of *S. lessoniana* collected from Japan and Thailand.

2. Materials and Methods

Sampling

Ninety individuals of the oval squid (*S. lessoniana*) originating from Rayong ($N=50$) located in the Gulf of Thailand and Nagasaki, Japan ($N=40$) were captured. Specimens from Thailand were kept at −70℃ and transported to Nagasaki University on dry ice. Oval squid from Nagasaki were captured locally and kept −80℃. At the Laboratory of Fisheries Biology, Faculty of Fisheries, Nagasaki University, liver tissue, mantle and buccal mass were dissected out from each squid from both populations and subjected to allozyme analysis.

Allozyme Analysis

Frozen tissue samples were homogenized mechanically in 0.25 ml of deionized water. Total solute proteins extracted from each individual were electrophoretically analyzed utilizing horizontal starch gel (11%) with 3 different buffer systems namely, C-A (pH 7.0), CAME (pH 6.8) and TC (pH 8.0) (SHAW and PRASAD, 1970; AEBERSOLD *et al.*, 1972; CLAYTON and TRETIAK, 1972). The starch gels were subsequently stained with 12 enzymes (Table 1).

Data Analysis

Banding patterns on the gels were interpreted as reflecting inherited Mendelian genetics predicted by the subunit composition of each enzyme (MAY, 1992). Observed and expected heterozygosity were calculated (NEI, 1987). The average effective number of alleles was examined according to CROW and KIMURA, (1965). Allele frequencies at each locus of each geographic samples were calculated and analyzed against Hardy-Weinberg equilibrium using goodness-of-fit (G) statistics. Genetic similarity and divergence were estimated using Roger's similarity index (R) (ROGER 1972) and Nei's genetic indentity (I) and distance (D) (NEI, 1987), respectively. Geographic heterogeneity in allele distribution frequencies between populations was also analyzed using the G statistic.

3. Results

Twelve enzymes were successfully resolved and used for analysis of allozyme polymorphism in Thai and Japanese *S. lessoniana*. These accounted for a total of 22 putative loci (Table 1). A fixed allele was observed in 50% of screened loci (*AK-3**, *AK-4**, *AK-5**, *ACP**, *DIA-6**, *DIA-3**, *G3PDH-1**, *G3PDH-2**, *IDHP-1**, *IDHP-2* and MDH-1**). Eleven loci (*MDH**, *PGDH**, *DIA-1**, *ATT**, *AK-1**, *AK-2* **, *ALP**, *MPI**, *GPI**, *PGM-1* and PGM-2**) were indicated. Polymorphism was estimated at 5% with the most common allele being less than 95% overall in investigated specimens. Seven, six and five polymophic allozymes could be detected in the buccal mass, muscle and liver tissue, respectively.

Good agreement between observed and expected heterozygosity of each *S. lessoniana* population suggested Hardy-Weinberg equilibrium in each population of *S. lessoniana* at all loci. All the examples were regarded as representing simple Mendelian populations. However, fixed alleles that can differentiate Thai and Japanese *S. lessoniana* populations were not observed. Generally, similar levels of allele frequencies were observed across all loci

Tabel 1. The numbers of alleles, protein structure, eletrophoretic buffer systems, and tissue sources of allozymes screened in this study.

Enzyme	Abbre viation	Tissue	Buffer System	No of Alleles	Protein Structure	D
Glycerol-3-phosphate dehydrogenase	*G3PDH-1**	M	3	1	Tetramer	>0.95
	*G3PDH-2**	M	3	1		
Malate dehydrogenase	*MDH-1**	M	1	1	Dimer	
	*MDH-2**	M	1	2	Dimer	>0.95
Isocitrate dehydrogenase	*IDHP-1**	M	1	1	Dimer	>0.95
	*IDHP-2**	M	1	1		
Phosphogluconate dehydrogenase	*PGDH**	B, L	1	3	Dimer	<0.95
Diaphorase	*DIA-1**	B, L	2	2	Monomer	<0.95
	*DIA-2**	B, L	2	1	Monomer	
	*DIA-3**	B, L	2	1	Monomer	
Aspatate amiotransferase	*AAT**	B, L	1	2	Dimer	<0.95
Adenylate kinase	*AK-1**	M	1	2	Monomer	<0.95
	*AK-2**	M	1	2	Monomer	<0.95
	*AK-3**	M	1	1	Monomer	>0.95
	*AK-4**	M	1	1	Monomer	>0.95
	*AK-5**	M	1	1	Monomer	>0.95
Alkaline phosphate	*ALP**	B, L	2	2	Monomer	<0.95
Acid phosphatase	*ACP**	M	3	1	Dimer	>0.95
Mannose phosphate isomerase	*MPI**	M	3	2	Monomer	<0.95
Glucose phosphate isomerase	*GPI**	B, L	1	3	Dimer	<0.95
Phosphoglucomutase	*PGM-1**	M, B	1	2	Monomer	<0.95
	*PGM-2**	M, B	1	2	Monomer	<0.95

Buffer system: 1=C-A pH 7.0, 2=CAME pH 6.8, 3=TC pH 8.0;
Tissues: M=mantle, B=buccal mass, l=liver
P=frequency of common allele for polymorphic locus.

except those of *GPI** and *MDH-2**, which showed significant differences between localities ($P<0.05$). The Thai squids showed low frequency of alleles a (0.11) and b (0.13) of each respective locus of *MDH-2** and *GPI** but these alleles were not detected in the Japanese *S. lessoniana* population (Table 2).

The average effective number of alleles per locus in *S. lessoniana* was 1.09. The percentage of polymorphic loci at 5% level and an average heterozygosity of the Japanese *S. lessoniana* were 45.45% and 0.28, whereas those of the Thai population was 50.00% and 0.23, respectively (Table 2). Both Nei's genetic indentity (I=0.997) and Roger's similarity index (R=0.973) were comparable. The genetic distance (D=−1n I) was 0.003 suggesting close genetic relationship between these populations. Geographic heterogeneity analysis did not indicate any difference in allele distribution frequency between the Japanese and Thai *S. lessoniana* for all loci ($P>0.05$).

Discussion

Population differentiation within a particular species is influenced by several factors such as, migration (or gene flow), random genetic drift, modes of natural selection, mutation and genetic recombination through mating systems. Additionally, biological factors related to individual groups of organisms for instance, ecological factors and life history (SEGAWA *et*

Tabel 2. Allele frequencies, percentage of polymorphic loci, effective number of alleles and observed heterozygosity of two conspecific populations of *S. lessoniana* orginating from Japan and Thailand based on allozyme analysis.

Locus	Allele	Geographic population	
		Japan (Nagasaki) (N=39–40)	Thai (Rayong) (N=25–50)
*AAT**	*a	0.885	0.837
	*b	0.115	0.163
*AK-1**	*a	0.461	0.227
	*b	0.538	0.773
*AK-2**	*a	0.526	0.660
	*b	0.474	0.340
*ALP**	*a	0.125	0.390
	*b	0.875	0.610
*DIA-1**	*a	0.387	0.357
	*b	0.612	0.643
*GPI**	*a	0.525	0.859
	*b	0	0128
	*c	0.475	0.013
*MDH-2**	*a	0	0.110
	*b	1	0.890
*MPI**	*a	0.500	0.437
	*b	0.500	0.562
*PGDH**	*a	0.075	0.033
	*b	0.862	0.7935
	*c	0.062	0.174
*PGM-1**	*a	0.475	0.462
	*b	0.525	0.537
*PGM-2**	*a	0.175	0.150
	*b	0.825	0.850
% Polymorphic loci		45.45	50.00
Effective number of alleles		1.09	1.09
Expected heterozygosity (Hobs)		0.179	0.183

N=number of individuals assayed.

al., 1993), also play a partitioning role in population differentiation (RYMAN and UTTER, 1987; AVISE, 1994).

There have been no publications estimating genetic variation levels in *S. lessoniana* in Thailand specifically. Whereas, some Japanese populations have been found to be quite variable genetically (IZUKA *et al.*, 1994; YOKOGAWA and UETA, 2000). Although the sample sizes used in this study were small, specimens were collected from two sampling sites located over thousands of kilometers in distance. Our preliminary results should be reliable for determination of genetic differentiation within this species at macrogeographic scales. Nevertheless, larger numbers of populatons should be collected before an unambiguous conclusion on intraspecific genetic differentiation can be drawn in *S. lessoniana* from populations in closer geographical proximity.

Information from Japanese fishermen reveals that there are groups of this species (AKAIKA, SHIROIKA and KUAIKA) distinguished by body color, shape and meat quality. Such differences in appearance have not been reported in Thailand. Allozyme studies on these groups suggest genetic differences at the subspecific rather than intraspefic level of a single species (IZUKA *et al.*, 1994). According to IZUKA *et al.* (1996), *S. lessoniana* (SHIROIKA) from Honshu, Shikoku, Ryukyu Islands and Thailand share a common gene pool over their 2,000 km geographical distance. This SHIROIKA group is a widely distributed subspecies in the tropical to warm temperate areas of the northwestern Pacific (IZUKA *et al.*, 1994). Although comparative morphology studies were not carried out, it may be possible that the SHIROIKA group is closely related to populations of *S. lessoniana* in Thailand. This is reflected in lack of genetic differentiation between this Japanese and Thai *S. lessoniana* revealed by allozyme polymorphism. These conclusions suggest that *S. lessoniana* may share a panmictic gene pool over a wide geographic area. Such patterns may have been mediated by a shared geological history governed by oceanic currents. The high % polymorphic loci and heterozygosity of *S. lessoniana* in this study reflects a large effective population size. Thus, localized genetic adaptation may take place with very little differentiation at neutral loci. The absence of obvious physical barriers between these two regions might have resulted in a high gene flow level of this species. Considering external morphology of *S. lessoniana*, the large conspicuous fin implies that it is a vagile species (ROPER *et al.*, 1984). Therefore, the Thai and some Japanese gene pools may have become homogenised by its migratory ability. Conformation of Hardy-Weinberg equlibrium

as revealed by non-significant difference between observed and expected heterozygosity of *S. lessoniana*, indicates that specimens used in this study were sampled from a large random mating population. However, a comparatively greater genetic differentiation of this squid between other locations in closer proximity (IZUKA *et al.*, 1996) could be due to smaller effective population sizes, local adaptation, less potential of gene flow and time of separation. Some groups of the species within Japanese waters seem to have barriers to panmixia. Most notables are oval squid populations surrounding the Ogasawara Islands and other sampling locatities. Is it possible that the Kuroshio Current can act as a natural barrier to migration? The oval squid populations in Japan have distinct differences in genetic distance values (YOKOGAWA and UETA, 2000). The squid populations in Japanese localities are probably under heavy fishing pressure. This is suggested by the relatively low average precentage of polymorphic allozyme loci and heterozygosity values.

The precentage of polymorphic allozyme loci in *S. lessoniana* in this study from both Royong and Nagasaki was quite high and comparable to that previously reported in the squids, *L. chinensis* (45.5–54.45%), *Ommastrephes batramii* 46%) and *Berryteuthis magister* (43%) (KATUGIN, 1993; YEATMAN and BENZIE, 1993). However, our reported values were much higher than that of other groups of *S. lessoniana* within the southwestern Japan complex (19.3%) (IZUKA *et al.*, 1996). Low percentage of polymorphic loci has also been reported for other *Loligo* species with values ranging from 0 to 29% (GARTHWAITE *et al.*, 1986; AUGUSTYN and GRANT, 1988; CARVALHO and LONEY, 1989; YEATMAN and BENZIE, 1993).

Apart from polymorphic loci, average heterozygosity of *S. lessoniana* reported in this study is also higher than that of the same species in southwestern Japan (H=0.037) (IZUKA *et al.*, 1996) and other loliginid species. Some examples are heterozygosity values reported for *L. plei* (H=0), *L. brevis* (H=0) and *L. pealei* (H=0.006), (GARTHWAITE *et al.*, 1986), *L. opalescence* (H=0.037) (AUGUSTYN and GRANT, 1988), *L. vulgaris reynandii* (H=0.030) and *L. gahi* (H=0.059) (CARVALHO and LONEY, 1989), *L. bleekeri* (H=0.003) (SUZUKI *et al.*, 1993), and *L. chinensis* (H=0.006～0.009) (YEATMAN and BENZIE, 1993).

The difference in genetic diversity in *S. lessoniana* detected by allozyme analysis reflects the potential of this simple approach for population genetic studies. Results may be applied to fisheries management regime of *S. lessoniana* at least on a macrogeographic scale. Therefore, more extensive surveys of genetic diversity and differentiation of this squid should be further carried out over other areas of its distribution. More emphasis could be placed on populations in closer proximity in Thailand. Additional molecular approaches, for example; random amplified polymorphic DNA (RAPD), restriction analysis of PCR-amplified mtDNA genes and single copy nuclear DNA polymorphism (scnDNA) can also be utilized to ensure more precise conclusions on oval squid stock structure.

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