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Allozyme comparison between Japanese and Chinese Limnetic Pearl Mussels

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The limnetic pearl mussel allelic constitution of 15 allozyme loci was compared between Japanese *Hyriopsis schlegeli* from Lake Biwa and Chinese *H. cumingi* from the Yang-ji-jiang River. They had no locus which was different and diagnostic between them. The genetic variability of *H. cumingi* was equal to other molluscs (percentage of polymorphic loci Pl = 26.7%, observed mean heterozygosity Ho = 0.098), whereas *H. schlegeli* exhibited reduced genetic variability (Pl = 6.7%, Ho = 0.013). *H. schlegeli* may have experienced a bottle neck effect or genetic drift in Lake Biwa. Nei's unbiased genetic distance between *H. schlegeli* and *H. cumingi* was quite low (D = 0.039～0.045) in spite of the great difference in shell morphology. The values corresponded to the inter-population level of North American unionid species.

1 Introduction

The unionid mussel genus *Hyriopsis* is famous as the source of limnetic pearls. The genus has two recognized species to the Far East : Japanese "Ike-chougai" *H. schlegeli* (v. Martens) endemic to Lake Biwa (Fig. 1A), and Chinese "Hire-ikechougai" *H. cumingi* (Lea) from the Yang-ji-jiang River system (Fig. 1B).[1] Although they have been cultured much in each country, their genetic variability and genetic relationships are still unknown to date.

This research presents allozyme comparison between *H. schlegeli* and *H. cumingi*, indicating that these two species are closely related each other in spite of their great shell shape difference: shells have high wings in *H. cumingi* (Fig. 1B) but not in *H. schlegeli* (Fig. 1A).[1]

2 Materials and methods

The cultured and wild Japanese *H. schlegeli*, and cultured Chinese *H. cumingi* were collected from 1993 to 1994. Collections sites, age and number of mussels are shown in Table 1.

The live mussels were frozen and stored at -70 ℃ at the laboratory until processed for horizontal starch-gel electrophoresis (12 % gel) and enzyme staining.[2－5] Table 2 indicates the 10 enzymes, 15 loci analyzed, source tissues and buffers utilized. Locus and gene nomenclature follows Shaklee et

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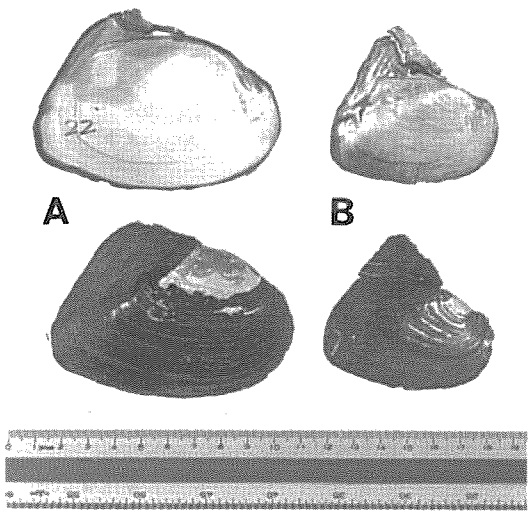

Fig. 1. Shells of Japanese *Hyriopsis schlegeli* from Lake Biwa (A) and Chinese *H. cumingi* from the Yang-ji-jiang River (B).

al.[8] Identical enzymes occupying different loci are numerically referenced from the anodal to cathodal position on the gel. The most common allele at a locus of *H. schlegeli* is designated as **100*.

3 Results and discussion

Four loci were polymorphic (Table 3): *PGM-2** in *H. schlegeli*, and *CAP-1**, *GPI**, *MDH-2**, and *PGM-2** in *H. cumingi*. The dominant alleles in *CAP-1**, *GPI**, and *MDH-2** were in common to the three populations. In *PGM-2**, the allele **112* was the most frequent in H. cumingi (0.368), but **100* was predominant in *H. schlegeli* (0.729 - 0.929) and rather rare in *H. cumingi* (0.092). The observed genotypic frequencies were not deviated from the values expected from the Hardy-Weinberg equilibrium with respect to these loci for all populations (χ^2-test, $p > 0.05$).

The genetic variability (Table 4) was low in *H. schlegeli* (6.7% polymorphic loci (Pl), 0.013 observed mean heterozygosity (Ho), and 0.013 expected mean heterozygosity (He)), and high in *H. cumingi* (26.7% Pl, 0.098 Ho, and 0.106 He). Ho/He value in *H. cumingi* (0.925) was lower than 1, which corresponds to the fact that the excess of homozygosity generally prevails in molluscs.[10] On the other hand, the values in *H. schlegeli*, 0.941 (wild) and 1.111

Table 1. Sample data of *Hyriopsis* used in this study

Sample	Locality	Age	Number of individuals
1. Japanese limnetic pearl mussel *H. schlegeli*	A culture farm in Lake Biwa Shiga Pref.	5 - 7	35
2. ibid.	Lake Biwa	more than 5	30
3. Chinese limnetic pearl mussel *H. cumingi*	A culture farm in Yang-ji-jiang River China	1	39

Table 2. Enzymes, enzyme numbers, loci, tissues, and buffer system used

Enzyme	Enzyme number	Locus	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1.	*AAT-1**	Adductor muscle	TC
Acid phosphatase	3.1.3.2.	*ACP**	Mid gut grand	AC
Cystosol aminopeptidase	3.4.11.1.	*CAP-1**	Am	RW
		*CAP-2**	Am	RW
Glycero-3-phosphate dehydrogenase	1.1.1.8.	*G3PDH**	Mgg	TC
Glucose-6-phosphate isomerase	5.3.1.9.	*GPI**	Am	RW
Isocitrate dehydrogenase	1.1.1.42.	*IDHP**	Mgg	TC
Malate dehydrogenase	1.1.1.37.	*MDH-1**	Am	TC
		*MDH-2**	Am	TC
		*MDH-3**	Am	TC
Phosphogluconate dehydrogenase	1.1.1.44.	*PGDH**	Am	TC
Phosphoglucomutase	5.4.2.2.	*PGM-1**	Am	RW
		*PGM-2**	Am	TC
		*PGM-3**	Mgg	RW
Superoxide dismutase	1.15.1.1.	*SOD**	Am	RW

TC: Tris-citrate buffer (pH 8.0, diluted 1 : 9 for the gel) by Shaw and Prasad[2], 4 mA/㎠ for 4 hr.

AC: Amine (N-(3-Aminopropyl)-morpholine) citrate buffer (pH 6.0) by Clayton and Tretiak[6], 4 mA/㎠ for 3 hr.

RW: Tris-citric acid (gel pH 8.5), lithium hydroxide-boric acid (tray pH 8.5) buffer system by Ridgway et al.[7], 4 mA/㎠ for 2 hr.

(cultured), were higher than in *H. cumingi*, which may be stochastic due to the low heterozygosity seen in *H. schlegeli*.

The genetic variability exhibited by *H. cumingi* was determined to be equal to other molluscs (e.g. two species of Japanese *Corbicula*, mean 0.117 Ho and 0.119 He[5], 11 species of American unionid, mean 0.078 He[11], 25 species of marine molluscs, mean 0.129 Ho and 0.147 He[10]), whereas *H. schlegeli* exhibited reduced genetic variability. Wild *H. schlegeli* may have experienced a hard bottle neck effect or genetic drift[12] in Lake Biwa as well as the cultured *H. schlegeli*, and have reduced their genetic variability.

Nei's unbiased genetic distance (D)[9] between *H. schlegeli* and *H. cumingi* were 0.045 (cultured *H. schlegeli* vs. *H. cumingi*) and 0.039 (wild *H. schlegeli* vs. *H. cumingi*). In spite of the great difference of shell morphology between *H. schlegeli* (with high shell wings) and *H. cumingi* (with low shell wings)[1], they had no locus which was almost different and diagnostic between them. The D values well corresponded to the inter-population level of a single species (0.010 - 0.184, mean 0.073), rather than the level scored among species (0.010 - 0.446, mean 0.211) of North American Unionidae, the genus *Elliptio*.[11] The values scored between *H. schlegeli* and *H. cumingi* are corresponded to about 0.2 million years ago by Nei's protein calibration.[12] However, there seems to be no evidence indicative of a direct freshwater connection between Lake Biwa and the Yang-ji-jiang River at that time.[13] No such animals have been known yet. More detailed future study on such as multilocus enzyme analysis, mtDNA comparison, etc. may resolve this paradox.

Table 3. Allele frequencies at polymorphic loci in 3 populations of *Hyriopsis*

	H. schlegeli		*H. cumingi*
Locus	1	2	3
CAP-1 *			
*106	0.000	0.000	0.346
*104	0.000	0.000	0.013
*100	1.000	1.000	0.590
*96	0.000	0.000	0.051
GPI			
*147	0.000	0.000	0.167
*100	1.000	1.000	0.833
MDH-2			
*122	0.000	0.000	0.064
*100	1.000	1.000	0.936
PGM-2			
*123	0.000	0.000	0.289
*112	0.071	0.150	0.368
*100	0.929	0.850	0.092
*89	0.000	0.000	0.250

Population numbers correspond to those in Table 1.

Table 4. Proportion of polymorphic loci and average heterozygosity (standard errors in parentheses) in 3 populations of *Hyriopsis*

Population	Percentage of loci polymorphic*	Mean heterozygosity	
		Observed	Expected**
H. schlegeli			
1.	6.7	0.010 (0.010)	0.009 (0.009)
2.	6.7	0.016 (0.016)	0.017 (0.017)
H. cumingi			
3.	26.7	0.098 (0.043)	0.106 (0.057)

Population numbers correspond to those in Table 1.
*: A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
**: Biased estimate[9].

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日本産イケチョウガイと中国産ヒレイケチョウガイのアロザイム比較

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淡水真珠母貝である日本琵琶湖産イケチョウガイ *Hyriopsis schlegeli* および中国長江産ヒレイケチョウガイ *H. cumingi* のアロザイム15遺伝子座における対立遺伝子組成と変異性を比較した。両種間で特徴的な分岐遺伝子座は認められなかった。ヒレイケチョウガイは，一般的な軟体動物とほぼ同程度の遺伝的変異性を示していた（多型遺伝子座率 Pl = 26.7%，平均ヘテロ接合体率観察値 Ho = 0.098)。一方，イケチョウガイでは変異性が低く（Pl = 6.7%，Ho = 0.013)，本種が瓶首効果や遺伝的浮動などによって遺伝的多様性を減じている可能性が示唆された。貝殻形態の大きな差異にもかかわらず，両種は遺伝的に比較的近く（遺伝的距離 D=0.039～0.045)，この値は，北米産イシガイ科の種内集団間レベルに一致していた。