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 PI = 26.7%, observed mean heterozygosity Ho = 0.098), whereas *H. schlegeli* exhibited reduced genetic variability (PI = 6.7%, Ho = 0.013). *H. schlegeli* may have experienced a bottleneck 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-chouglai" *H. schlegeli* (v. Martens) endemic to Lake Biwa (Fig. 1A), and Chinese "Hire-ikechouglai" *H. cumingi* (Lea) from the Yang-ji-jiang River system (Fig. 1B). 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).

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 °C at the laboratory until processed for horizontal starch-gel electrophoresis (12% gel) and enzyme staining. Table 2 indicates the 10 enzymes, 15 loci analyzed, source tissues and buffers utilized. Locus and gene nomenclature follows Shaklee et
Fig. 1. Shells of Japanese *Hyriopsis schlegeli* from Lake Biwa (A) and Chinese *H. cumingi* from the Yang-ji-jiang River (B).

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 (χ²-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 (Ho)), 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 mollusks. 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 | A culture farm in Lake Biwa | 5 - 7    | 35               |
| 2. *H. schlegeli*                | Shiga Pref.  |          |                  |
| 3. Chinese limnetic pearl mussel | A culture farm in Yang-ji-jiang River | 1        | 39               |
| 4. *H. cumingi*                  | China        |          |                  |
Allozyme of Limnetic Pearl Mussels

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

Nei's unbiased genetic distance (D) 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), 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*. The values scored between *H. schlegeli* and *H. cumingi* are corresponded to about 0.2 million years ago by Nei's protein calibration.

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. No such animals have been known yet. More detailed future study on such as multilocus enzyme analysis, mt-DNA comparison, etc. may resolve this paradox.

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### References

1) A. Machii: Pearl story, Shoukabou, Tokyo, 1995, p.191. (In Japanese.)

日本産イケチョウウガイと中国産ヒレイケチョウ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ウガイ Hyriopsis schlegeli および中国長江産ヒレイケチョウウガイ 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 P1 = 26.7%, 平均ヘテロ接合体率観察値 H0 = 0.098)。一方、イケチョウウガイでは変異性が低く (P1 = 6.7%, H0 = 0.013)。本種が群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。