Morphological and Genetic Differences between Japanese and Chinese Red Ark Shell *Scapharca broughtonii*

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(Received April 9, 1996)

Morphological and genetic differences between Japanese and Chinese red ark shell *Scapharca broughtonii* were examined. Differences were recognized between the two forms in all of the morphological characters examined. In particular the number of radial costae and shell weight index differed sufficiently for unequivocal differentiation of the forms, when used in combination. Isozyme analyses of genetic characters indicated differences in allelic frequencies at the *MEP-I**, *PGM** and *PROT** loci. The genetic distance (D value) between the Japanese and the Chinese forms, calculated from isozymic allele frequencies was 0.108, suggesting significant genetic independency of the two forms. The considerable morphological and genetic differences suggested that the Chinese red ark shell is a distinct subspecies or species from *Scapharca broughtonii*.

Key words: ark shell, *Scapharca broughtonii*, morphology, genetics, isozyme, China

Red ark shell *Scapharca broughtonii* (Schrenck) is one of the most important shellfishes in fisheries in Japan and adjacent countries. In Japan, its artificial culture has been vigorously made. Since demand for aquacultural seeds increased in recent years, the supply of the seeds is not always sufficient. In Kagawa Prefecture, about 850 thousand of wild red ark shell (Fig. 1) were imported from China in 1993–1994 to supplement the shortage of the seeds. And the imported Chinese red ark shell were released into the natural sea area for farming.

Consequently, the effects of the existence of free-living Chinese red ark shell in Japanese waters is of concern, necessitating examination of their biological features. For this reason, the present study was carried out to clarify morphological and genetic differences between the Japanese and the Chinese red ark shells.

Materials and Methods

Morphological Characters

Data from specimens used for morphological analyses are shown in Table 1. The red ark shell from Japan (hereafter called Japanese form) were collected from off Kwarazu, Ehime Prefecture and from off Shido and off Takuma, Kagawa Prefecture, all the places being located in the Seto Inland Sea. Total number of the specimens was 87.

The red ark shell from China (hereafter called Chinese form) were sampled from aquacultural seeds, which were transported from Dalian, Liaoning Province, China to Kagawa Prefecture in February, 1994. They were originally caught in the Bohai Sea. Total number of the specimens was 82.

As length-measured characters, shell length (SL), shell height (SH), shell depth (SD), hinge length (HL) and shell

weight (SW) were measured. Meristically, number of radial costae (RC) and number of hinge teeth (HT) of the left valve were counted. To express the shell weight relatively, the shell weight index (SWI) was defined by a formula as follows:

$$SWI = SW / SL^3 \cdot 10^5$$

Genetic Characters

Data from specimens used for genetic analyses are shown in Table 1. Japanese forms were 24 of the 34 specimens collected from off Kwarazu, which were used for the morphological analyses. Chinese forms were 24 of the 82 specimens used for the morphological analyses. The specimens were preserved in a refrigerator at either -80°C or -30°C .

Isozymes detected by horizontal starch-gel electrophoresis were used as genetic markers. Approximately 1 g of tissue was removed from the specimens in frozen condition, and it was put into a 1.5 ml micro tube. In case of the diges-

Fig. 1. General aspects of Chinese red ark shell with Japanese red ark shell.

A: Chinese form, SL 58.7 mm, SH 48.6 mm; B: Japanese form, SL 60.2 mm, SH 49.2 mm.

Table 1. Collection and treatment data of specimens

Morphological examinations						
	Japanese					Chinese
Month of collection	Feb. 1976	/	Mar. 1994	/	May 1994	Feb. 1994
Locality	Takuma	/	Kawarazu	/	Shido	Transported from Dalian
Number of individuals	24	/	34	/	29	82
Size range (SL, mm)	19.6–40.6	/	77.4–120.1	/	58.0–82.3	34.0–63.7
Average size (SL, mm)	27.3	/	109.8	/	66.9	52.9
Electrophoretic analyses						
	Japanese					Chinese
Month of collection	Mar. 1994					Feb. 1994
Locality	Kawarazu					Transported from Dalian
Number of individuals	24					24
Size range (SL, mm)	77.4–119.0					49.4–59.6
Average size (SL, mm)	108.1					53.9
Preservation	Frozen in -30°C					Frozen in -80°C

Table 2. Enzymes and protein examined, and tissues where they are detected

Enzyme or protein name	Enzyme number	Locus	Tissue
Aspartate aminotransferase	2.6.1.1	<i>AAT</i> *	Adductor Muscle
Catarase	1.11.1.6	<i>CAT</i> *	Adductor Muscle
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	<i>GAPDH</i> *	Adductor Muscle
Glucose-6-phosphate isomerase	5.3.1.9	<i>GPI</i> *	Adductor Muscle
Isocitrate dehydrogenase (NADP ⁺)	1.1.1.42	<i>IDHP</i> *	Adductor Muscle
Leucine aminopeptidase	2.4.11.	<i>LAP-1</i> *	Digestive Diverticulum
		<i>LAP-2</i> *	Adductor Muscle
Malate dehydrogenase	1.1.1.37	<i>MDH</i> *	Adductor Muscle
Malic enzyme (NADP ⁺)	1.1.1.40	<i>MEP-1</i> *	Digestive Diverticulum
		<i>MEP-2</i> *	Digestive Diverticulum
Mannose-6-phosphate isomerase	5.3.1.8	<i>MPI</i> *	Adductor Muscle
Phosphogluconate dehydrogenase	1.1.1.44	<i>PGDH</i> *	Adductor Muscle
Phosphoglucomutase	5.4.2.2	<i>PGM</i> *	Adductor Muscle
General protein		<i>PROT</i> *	Adductor Muscle

tive diverticulum tissue, the sample was directly homogenized, whereas for the adductor muscle tissue, the sample was homogenized with 100 μl of distilled water. Further, the samples were centrifuged by 15,000 rpm for 10 minutes under 0°C condition, the supernatant fluid was used for the electrophoresis.

The electrophoretic experiment methods were based on Taniguchi and Okada,¹⁾ using citric acid-aminopropylmorpholine buffer (pH 6.0) in each case. Enzymes and a protein detected by electrophoresis are shown in Table 2. Gene nomenclature followed Shaklee *et al.*,²⁾ the alleles are symbolized as relative mobility percentages compared with the most dominant alleles identified. In addition, capital alphabet signs are also given to the alleles.

Results

Morphological Characters

Results of the morphological examinations are shown in Table 3. High level of significant differences in proportion between the two forms were recognized in all of the characters. SH, SD and SWI of the Chinese form were fairly larger than those of the Japanese form (Table 3). These matters indicate that the shell shape of the Chinese form is

more global than the Japanese form, and the shell of the Chinese form is thicker than the Japanese form.

As for the meristic counts, the number of hinge teeth (HT) plainly increased associated with growth in the both forms (Fig. 2). Therefore, the average values and ranges of HT are not available to examine the difference between the two species (Table 3). Thus, regressions between SL and HT of the two forms were calculated. Powers regressions gave the highest correspondence to both the forms, being expressed by formulae as follows:

$$\begin{aligned}\text{Japanese form: HT} &= 11.952 \cdot \text{SL}^{0.410} \quad (r=0.942) \\ \text{Chinese form: HT} &= 15.702 \cdot \text{SL}^{0.318} \quad (r=0.511)\end{aligned}$$

On the two formulae obtained, a covariance analysis was made. The F value resulted 2.080, being significant at 1% level. That is, the regressions of the two forms are significantly different, according to the parameters of the formulae and the graph (Fig. 2), HT of the Chinese form are relatively less than that of the Japanese form.

Regarding the number of radial costae (RC), since it did not vary with growth unlike HT, the average numbers and ranges were available to examine the differences between the two forms (Table 3). Histograms of RC frequencies in the two forms are shown in Fig. 3. Both resulted in plain

Table 3. Proportional measurements and meristic counts of Japanese and Chinese forms with *t* values between both average values

	Japanese		Chinese		<i>t</i>
	Average	Range	Average	Range	
Shell height ¹	76.99	65.05–92.01	84.21	75.59–92.22	13.091***
Shell depth ¹	60.85	47.25–70.50	67.60	55.88–85.14	8.746***
Hinge length ¹	62.93	55.57–70.87	60.21	53.25–69.41	4.939***
Shell weight index	7.78	5.40–10.70	12.28	7.18–15.28	18.958***
Hinge teeth	67.58	38–94	55.45	48–64	6.856***
Radial costae	41.25	39–44	45.83	43–50	21.614***

¹ Percentage of shell length.

*** Significant at 0.1% level.

Fig. 2. Relationship between shell length and hinge teeth. Open circles—Japanese form, dark circles—Chinese form.

normal distributions and indicated clear differences in mode and range. The average values differing by nearly 5 between the two forms and slightly overlapping in the range of 43–44 (Fig. 3). Although the range of RC overlapped between the two forms, the combination with SWI separated the two forms completely (Fig. 4).

Genetic Characters

Allelic frequencies at 14 loci detected by electrophoresis with χ^2 heterogeneities between the two forms are shown in Table 4, with some electrophoregrams of remarkable isozymes illustrated in Fig. 5. In addition to these loci, presence of 11 loci could be presumed in the other enzymes and protein such as acid phosphatase (*ACP-1**, *ACP-2**, *ACP-3**), adenylate kinase (*AK**), creatine kinase (*CK**), esterase (*EST-1**, *EST-2**), glycerol-3-phosphate dehydrogenase (*G3PDH**), hemoglobin (*HB**), octanol dehydrogenase (*ODH**) and superoxide dismutase (*SOD**). However, the enzymes and the protein were not available because they did not show enough activity or focusing of the zymograms was deficient.

The fitness of the allelic frequencies in polymorphic loci, according to Hardy-Weinberg equilibrium, was examined by chi-square test. Because no χ^2 values were significant at the 5% level, the two forms were regarded as being originated from simple Mendelian populations respectively.

Regarding to heterogeneities between the groups, chi-square tests indicated significant differences in many of the

Fig. 3. Frequency distribution of radial costae. Open bars—Japanese form, dark bars—Chinese form. **Fig. 4.** Relationship between shell weight index and radial costae. Open circles—Japanese form, dark circles—Chinese form.

loci examined (Table 4). Some of these loci and alleles are described as follows.

Malic enzyme (MEP): The *MEP-1** locus of the Japanese form had a major allele of *100, and a minor one of *80, whereas the locus in the Chinese form was monomorphic, being entirely occupied by the *100 allele (Fig. 5; Table 4).

Phosphoglucuronate dehydrogenase (PGDH): The *PGDH** locus of the Japanese form had a major allele of *100 with a frequency of 0.917, and a minor one (*0), whereas in the Chinese form, the frequency of *100 allele 0.844, with minor allele of *0 and *200 (Fig. 5; Table 4).

Phosphoglucosmutase (PGM): At the *PGM** locus of the Japanese form, a major allele (*100) with a frequency

Table 4. Allelic frequencies of Japanese and Chinese forms with χ^2 heterogeneities between both frequencies

Locus	Allele	Frequency		χ^2 hetero	Locus	Allele	Frequency		χ^2 hetero		
		Japanese	Chinese				Japanese	Chinese			
<i>AAT</i> *	*0 C	0.000	0.029	4.691	<i>MDH</i> *	*170 B	0.083	0.000	4.364*		
	*-100 A	0.977	0.853			*100 A	0.917	1.000			
	*-200 B	0.023	0.118			*100 A	0.682	1.000			
<i>CAT</i> *	*100 A	0.875	0.979	4.276	<i>MEP-1</i> *	*80 B	0.318	0.000	15.771***		
	*75 B	0.104	0.021			<i>MEP-2</i> *	*-40 B	0.405		0.214	5.235
	*65 C	0.021	0.000				*-100 A	0.452		0.548	
<i>GAPDH</i> *	*120 B	0.021	0.021	0.000	<i>MPI</i> *	*-180 C	0.143	0.238	11.089*		
	*100 A	0.979	0.979			*100 A	0.438	0.167			
<i>GPI</i> *	*100 A	0.750	0.771	1.048		*95 B	0.479	0.667			
	*80 B	0.250	0.229			*50 C	0.083	0.167			
	*100 A	0.771	0.813			2.199	<i>PGDH</i> *	*200 C		0.000	0.094
*75 B	0.229	0.188	*100 A	0.917	0.844						
<i>IDHP</i> *	*-100 A	0.964	0.864	1.387	<i>PGM</i> *	*0 B	0.083	0.063	25.329***		
	*-160 B	0.036	0.136			*125 D	0.000	0.042			
<i>LAP-1</i> *	*115 C	0.021	0.022	3.123			*120 B	0.021		0.375	
	*100 A	0.771	0.761		*100 A		0.979	0.521			
	*90 B	0.208	0.196		*85 C		0.000	0.063			
	*70 D	0.000	0.022		*175 B		0.130	0.938			
<i>LAP-2</i> *					<i>PROT</i> *				38.996***		

* Significant at 5% level.
 ** Significant at 1% level.
 *** Significant at 0.1% level.

Fig. 5. Selected electrophoretograms of some loci in Japanese and Chinese forms.

of 0.979, and a minor one (*120) were found, whereas the Chinese form had the *100 allele as the major allele, with a frequency of 0.521, and minor ones of *120, *85 and *125 (Fig. 5; Table 4).

General protein (PROT): At the *PROT** locus of the Japanese form, the major allele of *100 had a frequency of 0.870, with a minor allele of *175. In contrast, in the Chinese form, the major and minor alleles were essentially reversed, with the major allele being *175, with a frequency of 0.938, and the minor one (*100) (Fig. 5; Table 4).

Of the other isozymes, significant differences in allelic frequencies between the two forms were found in the *MDH** and *MPI** loci (Table 4).

The genetic distance (D value)³⁾ between the two forms, calculated from isozymic allele frequencies was 0.108, at least significant at the intersubspecific level of general livings.⁴⁾

Discussion

According to the genetic analysis, the D value between the two forms was 0.108. Although the value was significant at the interspecific level of general livings,⁴⁾ it was somewhat lower than the general interspecific level of marine bivalves (intrageneric average 0.248–0.974) summarized by Oniwa and Kijima.⁵⁾ However, some of the D values shown by them are smaller than the value obtained in this study, such as 0.098 which is between *Crassostrea gigas* and *C. angulata*, and 0.079 which is between *Saccostrea commercialis* and *S. glomerata*. Therefore, it may be reasonable to regard the D value between the Japanese and the Chinese red ark shell being the interspecific level.

This corresponds well with the morphological divergence between the two forms (Table 3), at least the morphological data suggest that the Chinese form is a distinct subspecies or species from *Scapharca broughtonii*. Since it is somewhat difficult to determine the taxonomic grade in this moment, the Chinese form is here referred as *Scapharca* sp. temporarily until formally described. *Scapharca broughtonii* is supposedly widely distributed in coastal waters of the Japanese Archipelago, Korea, northern China and Taiwan.^{6–8)} This study showed that a distinct subspecies or species, formerly confused with *Scapharca broughtonii*, inhabits the Chinese coast.

Wang *et al.*⁷⁾ described so-called *S. broughtonii* in China, indicating HT as about 70 and RC as 42–48. Although HT can not be discussed because it changes with growth (Fig. 5), RC by their description correspond well with the result of *Scapharca* sp. in this study (Fig. 6). Further, its photograph shown is very similar with *S. sp.* (Fig. 1) rather than *S. broughtonii* in shape. The same, Zheng⁹⁾ described that from Zhejiang Province in China, showing RC also as 42–48 and close shell proportions to the results in this study.

While Qi *et al.*¹⁰⁾ also described the same species and indicated RC as 42. However, their description is doubtful because RC can be counted at least 44 from the plate they showed. Further, its photograph shown is similar with *S.*

sp., and the calculated proportion from their data shown also correspond well with *S. sp.* Therefore, their description might be based on Japanese descriptions on *S. broughtonii*.

Lee *et al.*^{*1} examined characteristics of Korean red ark shell from Chinhae Bay and Chinese one from Shandong Province with morphometrics and nuclear DNA analyses. They detected considerable morphological and genetic differences between the Korean and the Chinese forms as same as this study.

Since differences between the Japanese and the Korean forms have not been examined yet, it is uncertain whether these two forms are the same species or not. However, the capture locality of the Korean form examined in their study is faced Tsushima Channel that is close to Japanese Archipelago. According to a description of *S. broughtonii* in Korea, the Korean form is very similar with the Japanese form.¹¹⁾ Jo *et al.*¹²⁾ examined regression between SL and SH and that between SL and SD of the Korean ark shell from east coast of the Korean Peninsula, and showed close allometric values to the Japanese form examined in this study.

Although the information does not always mean that an uniform species distributes around Korean Peninsula, at least some Korean populations like that examined by Lee *et al.*^{*1} and Jo *et al.*¹²⁾ may be the same species as the Japanese form. Suppose this was true, the fact that there were pronounced differences between the Korean and the Chinese forms was reasonable.

There are many living species which is supposed to broadly distribute around east Asian waters. However, populations which inhabit in the long distance like that between Japan and China can not genetically interchange. In the situation, lapse of historical time makes genetic divergence, consequently specific divergence can occur.

Actually, a marine fish of sea bass *Lateolabrax japonicus* has been supposedly widely distributed in east Asian coastal waters. However, Yokogawa and Seki¹³⁾ examined morphological and genetic differences between the Japanese and the Chinese sea bass, and concluded that the Chinese one is a different species from *L. japonicus* as same as the Chinese red ark shell in this study. The same, Ozawa and Tomida¹⁴⁾ made morphological comparison between Japanese and Chinese top shell *Turbo (Batillus) cornutus*, they recognized the Chinese top shell as a distinct species from the Japanese one, and described the Chinese top shell as *T. chinensis*. These examples of specific divergences might be natural because the Japanese and the Chinese populations never have genetic interchange.

Not only these matters, there can be many unknown living species which is confused in the known species. Descriptions of species tend to rely on literature and reports, this can cause the taxonomic confusion. Hereafter, the morphological and genetic examinations for the actual specimens should be made to clarify the present classifications.

Actually, not only the red ark shell but also many kinds of foreign fishes and shellfishes have been vigorously introduced into Japan as aquacultural seeds.^{*2} And some

*1 Lee *et al.*: Abst. 1st Metg. Korea-Japan Aquacult. Sci. Sympo. May, 1994, p. 154–156 (in Korean).

*2 Yokogawa: Abst. 1st Metg. Korea-Japan Aquacult. Sci. Sympo. May, 1994, p. 36–37 (in Japanese).

species of the foreign seeds genetically differed from Japanese native species.^{13,15,16)*3,*4} In case of the Chinese red ark shell, it must be a great problem that the seeds were released into the open sea. The free-living individuals will grow up and mature, they may participate the propagation with the Japanese ones. It can cause hybridization between the two forms. If the F₁ hybrids can propagate, the exotic gene will flow into the Japanese native gene resources, and that status can not return to the original condition by any means.

Therefore, in order to conserve the Japanese native genetic resources, it would be recommended that new introductions of the foreign seeds should be made with enough caution from now on.

Acknowledgments The author is very grateful to Mr. Ken-ichi Yamaga, Kagawa Prefectural Fisheries Experimental Station, who motivated to make this study and willingly offered the Chinese red ark shell specimens. Mr. Shinji Hirata, Ehime Prefectural Chūyo Fisheries Experimental Station, Mr. Fusanori Yakushiji, Tōyo branch of Ehime Prefectural Chūyo Fisheries Experimental Station, Mr. Chiyoteru Shimokawa, Fisheries Division of Kagawa Prefectural Government and Dr. Sadaaki Yoshimatsu, Kagawa Prefectural Red Tide Institute, assisted in collecting samples of the Japanese red ark shell specimens. Dr. Yasuo Kondo, Faculty of Science, Kōchi University and Mr. Ken-ichi Nakao, Tokushima Prefectural Museum, provided some literature. Dr. Shi Dong, Faculty of Agriculture, Kōchi University, assisted in translating Chinese literature.

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*3 Furukawa et al.: Abst. Metg. Japan. Soc. Fisheries Sci., October, 1993, p. 91.

*4 Yokogawa: Abst. 2nd Japan-Korea Joint Metg. Sympo. Aaquacult. July, 1996, pp. 33-34 (in Japanese).