Short Paper

Analysis of the Population Structure of the Swimming Crab, *Portunus trituberculatus* in the Coastal Waters of Okayama Prefecture, by RFLPs in the Whole Region of Mitochondrial DNA

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The swimming crab *Portunus trituberculatus* is widely distributed in the coastal waters of Japan. This species is of commercial importance in the coastal fisheries of Japan, yielding about 4,150 tons per year.¹⁾ Artificial propagation and stocking of this species began in 1960 under the auspices of the Ministry of Agriculture and Fisheries. The population structure of the swimming crab has been investigated previously by allozyme analysis.²⁾ Recently, analysis of restriction fragment length polymorphisms (RFLPs) of the whole region³⁾ and the D-loop region⁴⁾ of mitochondrial DNA (mtDNA) were performed to clarify the genetic variability and population structure. However, intra- and inter-population variability, success and the effects of stocking on the genetic composition of native populations have not yet been addressed.

In the present study, the genetic variability of the swimming crab at four sampling sites in Okayama Prefecture was studied using RFLPs analysis of the whole region of mtDNA.

In 1997, 511 individuals were collected by trawl fishing or gill net at four locations in Okayama Prefecture, Japan. The four sampling sites are Mizushima-Nada, Harima-Nada Northwest, Ushimado Town and Hinase Town (Fig. 1). These sites are located within about 100 kilometers of each other. The 4th legs from each specimen were frozen and shipped at -20°C for the test.

Total crude DNA was extracted by the method described previously.³⁾ The endonucleases, *Hinc* II, *Hpa* I and *Stu* I (Takara Shuzo Co., Japan) were selected for this study based on the results of a preliminary test showing polymorphisms in restricted fragment sizes. Polymorphisms were identified by agarose gel electrophoresis of the cleared mtDNA fragments. Electrophoretic patterns were visualized by southern blotting followed by DNA hybridization with digoxigenin labeled mtDNA of the swimming crab.³⁾ Mitochondrial DNA used as the probe was purified from a fresh, immature ovary of the swimming crab by CsCl-ethidium bromide density gradient ultracentrifugation.³⁾ Restriction patterns observed in each endonuclease digestion were alphabetically labeled and composited for comparing haplotype frequencies between the population samples. The nucleotide diversity⁵⁾ and haplotypic diversity^{5,6)} were calculated from the presence/absence matrix of restriction sites in each endonuclease

and frequency of each haplotype. The distribution of haplotypes between population samples was evaluated for homogeneity using the chi-square randomization method (Monte Carlo simulation)⁷⁾ with 10,000 randomizations of the data.

RFLPs profiles observed by each of the three endonucleases are shown in Table 1. Six RFLPs patterns were observed in *Hinc* II, four patterns in *Hpa* I, and four patterns in *Stu* I. The size of mtDNA in the swimming crab was estimated to be $15,961 \pm 290$ base pairs. Sixteen haplotypes were identified in the 511 individuals analyzed (Table 2). Haplotypes 1 to 3 were found in high frequencies in all the samples analyzed.

Genetic variability within the population samples was examined by the haplotypic diversity (Table 2) and nucleotide diversity (Table 3). Both estimates of genetic diversity indicate that all of the population samples analyzed have the same level of genetic variability.

We examined the degree of variability among the population samples. Estimates of nucleotide divergence among the population samples are shown in Table 3. The values were similar among all the combinations, ranging from 0.34 to 0.39%. However, as shown in Table 4, a significant level of heterogeneity was found between the population samples from Mizushima-Nada and those from Hinase Town ($p < 0.05$). Significant heterogeneity was also observed between the Mizushima-Nada and the other three popula-

Fig. 1. Location of sampling sites.

1: Mizushima-Nada ($n=287$: avg. long carapace width (LCW) = 184.9 mm). 2: Ushimado Town ($n=77$: LCW = 217.3 mm). 3: Hinase Town ($n=101$: LCW = 142.4 mm). 4: Harima-Nada Northwest ($n=46$: LCW = 153.1 mm).

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Table 1. Fragment size estimates (in base pairs) for three endonucleases that produced polymorphic restriction patterns in swimming crab mitochondrial DNA

	<i>Hinc</i> II						<i>Hpa</i> I				<i>Stu</i> I			
	A	B	C	D	E	F	A	B	C	D	A	B	C	D
6100	6100	6100	6100	6100	6100	—	14400	—	—	—	7600	—	—	—
4800	—	—	—	—	—	—	—	13200	—	—	—	7000	—	—
—	4200	—	—	—	—	—	—	—	13000	—	—	5500	5500	5500
—	—	—	—	—	—	3800	—	—	—	11500	—	—	4600	—
—	—	3600	—	—	—	—	—	—	—	3100	3650	—	3650	3650
3000	3000	3000	3000	3000	3000	3000	1900	1900	1900	1900	3500	—	—	3500
—	—	—	2800	—	—	2800	—	—	1500	—	—	2200	2200	2200
—	—	—	—	—	2400	—	—	1100	—	—	1200	1200	—	1200
—	—	—	—	2100	—	—	—	—	—	—	—	—	—	—
—	—	—	2000	2000	2000	—	—	—	—	—	—	—	—	—
1800	1800	1800	1800	1800	1800	1800	—	—	—	—	—	—	—	—
—	—	1200	—	—	—	—	—	—	—	—	—	—	—	—
—	600	—	—	600	—	—	—	—	—	—	—	—	—	—
Sum*	15700	15700	15700	15700	15600	15800	16300	16200	16400	16500	15950	15900	15950	16050

* Sums of the fragments are presented in the bottom row. (—: not present)

Table 2. Distribution of mtDNA haplotypes and haplotypic diversity of the swimming crabs sampled from Okayama Prefecture

Haplotype	Restriction morph			Mizushima-Nada	Ushimado Town	Hinase Town	Harima-Nada Northwest	Total
	<i>Hinc</i> II	<i>Hpa</i> I	<i>Stu</i> I					
1	D	A	D	140	33	36	19	228
2	D	D	D	75	28	26	12	141
3	A	A	D	52	10	28	12	102
4	D	A	C	3	0	0	0	3
5	D	A	A	4	1	5	0	10
6	E	A	D	6	0	1	0	7
7	D	D	A	1	2	1	0	4
8	B	A	D	2	0	0	0	2
9	D	A	B	1	2	1	0	4
10	C	C	D	1	0	0	0	1
11	D	C	D	1	0	0	0	1
12	A	A	A	1	0	0	0	1
13	E	B	D	0	0	1	1	2
14	B	B	D	0	0	2	1	3
15	F	A	D	0	0	0	1	1
16	C	B	D	0	1	0	0	1
Number of haplotypes				12	7	9	6	16
Total no. of specimens				287	77	101	46	511
Haplotypic diversity				0.622	0.674	0.734	0.707	0.694*

* Average of haplotypic diversity.

Table 3. Estimates of nucleotide divergence of the swimming crab

	Mizushima-Nada	Ushimado Town	Hinase Town	Harima-Nada Northwest
Mizushima-Nada	0.0032	0.0034	0.0037	0.0035
Ushimado Town		0.0034	0.0039	0.0038
Hinase Town			0.0041	0.0038
Harima-Nada Northwest				0.0038

Above the diagonal: between population samples.
Diagonal: within population samples.**Table 4.** Pairwise haplotype frequency comparisons based on chi-square randomization test, using the Monte Carlo simulation method

	Mizushima-Nada	Ushimado Town	Hinase Town	Harima-Nada Northwest
Mizushima-Nada		0.100 (0.003)	0.027* (0.002)	0.088 (0.003)
Ushimado Town			0.067 (0.002)	0.119 (0.003)
Hinase Town				0.783 (0.004)
Harima-Nada Northwest				

Numerals shown above and below the diagonal: p -values between each set of localities were calculated with 10,000 replications.

Below the diagonal: between the Mizushima-Nada and the other three population samples (pooled).

The standard errors shown in parentheses were obtained by the Monte Carlo simulation method of Roff and Bentzen (1989).

* Significant level at $P < 0.05$.** Significant level at $P < 0.01$.

tion samples, pooled ($p < 0.01$). Thus, the population at Mizushima-Nada could be distinguished from the populations at the other three locations by haplotype frequency.

In previous reports based on allozyme research, it has been proposed that the swimming crab²⁾ and the blue crab⁸⁾ have panmixial population structures throughout their wide distribution ranges. The present data suggests populations of the swimming crab are genetically heterogenous even in a small area such as the coastal waters of Okayama Prefecture.

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