Genetic differentiation among local populations of common freshwater shrimp *Paratya compressa improvisa*

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ABSTRACT

Nei's (1972) genetic distance were estimated among 21 populations of the common freshwater shrimp *Paratya compressa improvisa*, living in the northern part of Japan. The estimates were based on examination of 18 enzyme loci by starch-gel electrophoresis. These shrimp populations were genetically classified into three geographic groups on the basis of predominating alleles typical for two loci *Fdp-1* and *Fdp-2*. The "Kanto group" consisted of individuals having *Fdp-1a* and *Fdp-2c*, the "Japan Sea group" having *Fdp-1b* and *Fdp-2a*, and the "Pacific Ocean group" having *Fdp-1b* and *Fdp-2c*. Nei's genetic distance among the three different geographic groups, varied from 0.0572 to 0.2136. Average genetic distances were 0.1001 between Pacific Ocean and Japan Sea groups, 0.0851 between Pacific Ocean and Kanto groups, and 0.1990 between Japan Sea and Kanto groups. These values of genetic distance among the three groups are comparable with those among different subspecies.

1. INTRODUCTION

Geographic isolation is almost invariably necessary for speciation to occur (Mayr, 1942, 1963). Numerous biologists have reported the evidence for geographic speciation in many organisms from their data of geographic variation. The occurrence of differences among spatially separated populations of species is called geographic variation. Geographic variation is usually most apparent, when specimens from geographically far-distant populations are compared. Genetic difference of populations in different localities is an example of geographic variation. The previous paper (Ikeda et al., 1992) revealed that Nei's genetic distance between two *Paratya* subspecies, *P. compressa compressa* living in the southern part and *P. c. improvisa* living in the northern part of Japan, was 0.597, based on 18 enzyme loci by starch-gel electrophoresis.

The aim of the present study is to estimate the degree of genetic differentiation among geographic populations of common freshwater shrimp, *P. c. improvisa* living mainly in the northern part of Japan except for Hokkaido.

2. MATERIALS AND METHODS

Specimens of *P. c. improvisa* were collected from 21 different locations in the northern part of Japan except in Hokkaido during February–October in 1990 and 1991 as shown in Fig. 1. Specimens were transported to our laboratory alive or frozen at -18°C . Samples were stored in the laboratory at -80°C until elec-

Fig. 1. Map showing collection sites of *Paratya compressa improvisa*. Aomori Prefecture: 1=Shimizu River, 2=Yamazaki-tsutsumi Pond; Iwate Prefecture: 3=Yagisawa-tsutsumi Pond; Akita Prefecture: 4=Oh-numa Marsh, 5=Oh-tsutsumi Pond, 6=Shirahata-tsutsumi Pond; Yamagata Prefecture: 7=Kami-ike Pond, 8=Dojo-numa Marsh; Miyagi Prefecture: 9=Hinata-ike Pond, 10=Kamitomita-ike Pond, 11=Kitakami River, 12=Ushiami-ike Pond, 13=Imosawa Pond, 14=Kenmin-no-mori Pond, 15=Dainohara Pond; Fukushima Prefecture: 16=Oguni-numa Marsh, 17=Ukedo River, 18=Takase River, 19=Same River; Gunma Prefecture: 20=Lake Haruna; Saitama Prefecture: 21=Ranzan Pond. A dotted line outlines the geographic range of *P. c. improvisa* described by Kamita (1970). A: Kitakami River, B: Natori-Hirose River, C: Abukuma River.

trophoretically analyzed. The methods of electrophoresis have been described in the previous paper (Ikeda et al., 1992). The surveyed fifteen enzymatic proteins were as follows; aspartate aminotransferase (AAT; E.C. No.2.6.1.1), aldehyde dehydrogenase (ALDH; E.C. No. 1.2.1.5.), alkine phosphatase (ALP; E.C. No. 3.1.3.1), aldehyde oxidase (AO; E.C. No. 1.2.3.1), esterase (EST; E.C. No. 3.1.1.-), fructose-1,6-diphosphatase (FDP; E.C. No. 3.1.3.11), glycerol-3-phosphate dehydrogenase (G3PDH; E.C. No. 1.1.1.12), glucose-6-phosphate isomerase (GPI; E.C. No. 5.3.1.9), isocitrate dehydrogenase (IDHP; E.C. No. 1.1.1.42), lactate dehydrogenase (LDH; E.C. No. 1.1.1.27), maleate dehydrogenase (MDH; E.C. No. 1.1.1.37), malic enzyme (ME; E.C. No. 1.1.1.40), mannose-6-phosphate isomerase (MPI; E.C. No. 5.3.1.8), phosphogluconate dehydrogenase (PGDH; E.C. No. 1.1.1.44), phosphoglucomutase (PGM; E.C. No.5.4.2.2). In the case of two zones of activity in electrophoretic pattern on a gel, we estimated at least two different loci based on the following facts; 1) variation is observed in each zone, 2) heat stability difference is shown between two zones when the sliced gel was subjected to the heat treatment in the water bath. Alleles at each locus were designated by letters in alphabetical order, starting with the allele encoding the most anodally migrating isozymes. Expected heterozygosity (h_e) was estimated by $h_e = 2n(1 - \sum X_i^2)/(2n - 1)$, where n is the number of sampled individuals and X_i is the frequency of i -th allele at each locus. Average heterozygosity (H_e) was calculated as $H_e = \sum h_e / r$, where r is the number of loci examined. Nei's genetic distance (Nei, 1972), inbreeding coefficient within- and between-neighborhood in a hierarchical island model (Slatkin and Voelem, 1991) were calculated by using allele frequencies of 21 natural populations.

3. RESULTS

Polymorphism

A total of 18 enzyme loci of shrimp collected from 21 locations were examined by starch-gel electrophoresis. Of the loci examined, six loci (*Aldh*, *Alp*, *Ao*, *Est*, *Fdp-1*, and *Ldh*) were monomorphic in all populations. Nine loci (*Aat-1*, *Fdp-2*, *G3pdh*, *Gpi*, *Mdh*, *Me*, *Mpi*, *Pgdh*, and *Pgm*) were polymorphic in at least one population. A locus was considered as polymorphic if the frequency of the most common allele was no greater than 0.95. At the other three loci (*Aat-2*, *Idhp-1*, and *Idhp-2*) rare variants segregated in at least one population.

Allele frequencies at 10 loci are shown in Table 1. At all loci examined, the observed and the expected numbers under Hardy-Weinberg equilibrium of genotypes were in good agreement with each other. To estimate genetic variability, the proportion of polymorphic loci and average heterozygosity were calculated as shown in Table 2. The proportion of polymorphic loci ranged from 0.000 to 0.167 with an average of 0.084 over all populations, and the average heterozygosity

Table 1. Allele frequencies at 10 loci in 21 populations in *P. c. improvisa*

Locus	Allele	Pacific Ocean group										Japan Sea group					Kanto group						
		2 (50)	3 (50)	9 (57)	10 (36)	11 (50)	12 (92)	13 (50)	15 (105)	15 (64)	16 (50)	17 (52)	18 (96)	19 (50)	1 (31)	4 (50)	5 (50)	6 (50)	7 (50)	8 (36)	20 (27)	21 (48)	
<i>Aat-1</i>	<i>a</i>	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.714	0.880	1.000	1.000	1.000	1.000	1.000	1.000
	<i>b</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.286	0.120	0	0	0	0	0	0
<i>Fdp-1</i>	<i>a</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.000	1.000	
	<i>b</i>	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0	
<i>Fdp-2</i>	<i>a</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1.000	0.990	1.000	1.000	0.990	1.000	0	0	
	<i>b</i>	0	0	0	0	0	0.080	0.192	0	0	0	0	0	0	0	0.010	0	0	0.010	0	0	0	
<i>G3pdh</i>	<i>c</i>	1.000	1.000	1.000	1.000	1.000	1.000	0.920	0.808	1.000	1.000	1.000	1.000	1.000	0	0	0	0	0	0	1.000	1.000	
	<i>a</i>	0.010	0	0.009	0	0	0.010	0	0	0.090	0.010	0.005	0.090	0	0	0	0	0	0	0	0	0	
<i>Gpi</i>	<i>b</i>	0.990	1.000	0.991	1.000	1.000	1.000	0.990	1.000	1.000	0.910	0.990	0.995	0.910	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
	<i>a</i>	0	0	0	0	0	0	0	0	0	0.048	0	0	0	0	0	0	0	0	0	0	0	
<i>Mdh</i>	<i>b</i>	0	0	0.764	0.847	0.610	0.902	0.860	0.500	0.016	1.000	0.952	0.990	0.970	0	0	0	0	0	0	1.000	1.000	
	<i>c</i>	1.000	1.000	0.175	0.153	0.380	0.098	0.140	0.490	0.492	0	0	0.010	0.030	1.000	1.000	1.000	1.000	0.930	0.986	0	0	
<i>Me</i>	<i>d</i>	0	0	0.061	0	0.010	0	0	0.010	0.492	0	0	0	0	0	0	0	0	0	0	0	0	
	<i>e</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.070	0.014	0	0	
<i>Mpi</i>	<i>a</i>	0	0	0.100	0	0.100	0	0	0	0	0	0.135	0.266	0	0	0	0	0	0	0	0	0	
	<i>b</i>	1.000	1.000	0.900	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.865	0.734	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
<i>Pgdh</i>	<i>a</i>	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.980	0.984	0.940	0.520	1.000	0.940	0.889	0.981	1.000	
	<i>b</i>	0	0	0	0	0	0	0	0	0	0	0	0	0.020	0.016	0.060	0.480	0	0.060	0.111	0.019	0	
<i>Pgm</i>	<i>a</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0.371	0.170	0.050	0	0	0	0	0	
	<i>b</i>	1.000	1.000	0.991	0.930	0.990	1.000	0.940	0.770	0.789	1.000	0.952	0.906	1.000	0.629	0.830	0.950	1.000	1.000	1.000	0.788	0.458	
<i>Pgm</i>	<i>c</i>	0	0	0	0.042	0	0	0	0	0	0	0.048	0	0	0	0	0	0	0	0	0.222	0.542	
	<i>d</i>	0	0	0.009	0.028	0.010	0	0.060	0.230	0.211	0	0	0.094	0	0	0	0	0	0	0	0	0	
<i>Pgm</i>	<i>a</i>	0	0	0	0	0	0	0.020	0	0	0	0	0	0	0.048	0	0	0	0	0.014	0	0	
	<i>b</i>	1.000	1.000	1.000	1.000	1.000	1.000	0.980	1.000	1.000	1.000	1.000	1.000	0.820	0.952	1.000	1.000	1.000	0.960	0.861	1.000	1.000	
<i>Pgm</i>	<i>c</i>	0	0	0	0	0	0	0	0	0	0	0	0	0.180	0	0	0	0	0.040	0.125	0	0	
	<i>a</i>	0	0	0	0	0	0	0	0	0	0	0	0.015	0	0	0.070	0.020	0	0	0	0	0.073	
<i>Pgm</i>	<i>b</i>	1.000	1.000	1.000	1.000	0.990	0.967	1.000	0.990	1.000	1.000	0.981	0.980	1.000	1.000	0.930	0.980	1.000	0.990	0.990	1.000	0.927	
	<i>c</i>	0	0	0	0	0.010	0.033	0	0.010	0	0	0.019	0.005	0	0	0	0	0	0.010	0.010	0	0	

Sample size is in parentheses under each location number (see Fig. 1)

Aldh, *Alp*, *Ao*, *Est* and *Ldh* were monomorphic in all populations, and *Aat-2*, *Idhp-1* and *Idhp-2* showed rare variant alleles in at least one population.

Table 2. Proportion of polymorphic loci (P) and average heterozygosity (H_e) in *P. c. improvisa* populations

Group	Location	Sample size	No. of loci	P	H_e ($\pm$ SE)
Pacific Ocean group	2	50	18	0.000	0.001 ($\pm$ 0.003)
	3	50	18	0.000	0.000 ($\pm$ 0.000)
	9	57	18	0.056	0.026 ($\pm$ 0.021)
	10	36	18	0.111	0.022 ($\pm$ 0.016)
	11	50	18	0.056	0.032 ($\pm$ 0.027)
	12	92	18	0.056	0.016 ($\pm$ 0.010)
	13	50	18	0.167	0.031 ($\pm$ 0.016)
	14	105	18	0.167	0.069 ($\pm$ 0.036)
	15	64	18	0.111	0.050 ($\pm$ 0.033)
	16	50	18	0.056	0.009 ($\pm$ 0.009)
	17	52	18	0.056	0.026 ($\pm$ 0.014)
	18	96	18	0.111	0.034 ($\pm$ 0.023)
	19	50	18	0.056	0.031 ($\pm$ 0.018)
Japan Sea group	1	31	18	0.056	0.033 ($\pm$ 0.026)
	4	50	18	0.222	0.053 ($\pm$ 0.027)
	5	50	18	0.167	0.047 ($\pm$ 0.030)
	6	50	18	0.000	0.000 ($\pm$ 0.000)
	7	50	18	0.111	0.021 ($\pm$ 0.010)
Kanto group	8	36	18	0.111	0.023 ($\pm$ 0.015)
	20	27	18	0.111	0.027 ($\pm$ 0.019)
	21	48	18	0.056	0.033 ($\pm$ 0.026)
Average (Range)				0.084 (0.000–0.167)	0.028 (0.000–0.069)

Each location number is identical to that in Fig. 1.

ranged from 0.000 ($\pm$ 0.000) to 0.069 ($\pm$ 0.036) with an average of 0.028 over all populations. In order to test the differences of allele frequencies between populations, t -test were carried out. Significant differences between populations were observed at one or more loci ($P < 0.05$), except for a pair (Yamazaki-tsutsumi Pond and Yagisawa-tsutsumi Pond) which showed no polymorphic loci. From the results, each population was considered to be a breeding population isolated from the others.

Geographic differentiation

At 16 loci, the most frequent allele was the common one in all populations. At the remaining two loci (*Fdp-1* and *Fdp-2*), an allele predominated in some populations, while the other allele predominated in the other populations (divergent loci). The 21 populations were classified into three groups, namely the Pacific Ocean group, the Japan Sea group, and the Kanto group, according to their

Fig. 2. Electropherogram of fructose-1,6-diphosphatase (FDP) in *Paratyia compressa improvisa*. Genotypes are shown below the respective banding patterns.

Fig. 3. Geographic distribution of alleles at the two FDP loci, *Fdp-1* (left) and *Fdp-2* (right). I=Pacific Ocean group, II=Japan Sea group, III=Kanto group.

predominating alleles typical for two loci, *Fdp-1* and *Fdp-2* (Fig. 2). As shown in Fig. 3, the Kanto group consisted of individuals having *Fdp-1a* and *Fdp-2c* but not *Fdp-1b*, *Fdp-2a*, and *Fdp-2b*. The Japan Sea group included not *Fdp-1a* and *Fdp-2c* but *Fdp-1b* and *Fdp-2a*, and had *Fdp-2b* at a moderate frequency in the populations, the Pacific Ocean group, not *Fdp-1a* and *Fdp-2a* but *Fdp-1b* and *Fdp-2c*, and *Fdp-2b* at a moderate frequency.

The inbreeding coefficient within- (F_{SN}) and between-neighborhood (F_{NT}) were calculated as shown in Table 3. F_{NT} displayed higher values (0.6494–0.7623) than those of F_{SN} (0.1329–0.3906). From these values, the product of effective size of population and migration rate ($N_e \times m$) within and between neighborhood were also calculated. These results indicated that $N_e \times m$ within neighborhood were much larger than between neighborhood.

Table 3. Inbreeding coefficient within- and between-neighborhood. F_{SN} =within-neighborhood inbreeding coefficient; F_{NT} =between-neighborhood inbreeding coefficient; m =migration rate; N_e =effective size of population

Group	Number of populations	F_{SN} (F_{NT})	$N_e \times m$
Pacific Ocean	13	0.3906	0.3324
Japan Sea	6	0.1652	0.8773
Kanto	2	0.1329	0.4078
Pacific Ocean + Japan Sea	19	0.6494	0.0337
Pacific Ocean + Kanto	15	0.7623	0.0195
Japan Sea + Kanto	8	0.7028	0.0263
Total	21	0.6786	0.0263

Table 4. Nei's genetic distance (mean value) of intra- and intergroups of *P. c. improvisa* populations

	Pacific Ocean group	Japan Sea group	Kanto group
Pacific Ocean group	0.0195 (78, 0.0000–0.0579)	—	—
Japan Sea group	0.1001 (78, 0.0572–0.1431)	0.0083 (15, 0.0004–0.0196)	—
Kanto Group	0.0851 (26, 0.0617–0.1394)	0.1990 (12, 0.1852–0.2136)	0.0063 (1)

Numbers in parentheses represent the number of compared pairs and the ranges of Nei's genetic distance.

Nei's genetic distance (D) was calculated for each pair of local populations (Table 4). Within the Pacific Ocean group, D values varied from 0.0000 to 0.0579 with a mean of 0.0195; within the Japan Sea group, D values ranged from 0.0004 to 0.0196 with a mean of 0.0083; within the Kanto group (two populations), D value was 0.0063. Mean D values and ranges (in parentheses) among the three groups were as follows; 0.1001 (0.0572–0.1431) between the Pacific Ocean group and the Japan Sea group, 0.0851 (0.0617–0.1394) between the Pacific Ocean group and the Kanto group, and 0.1990 (0.1852–0.2136) between the Japan Sea group and the Kanto group. The distance tree drawn by the neighbor-joining method (Saitou and Nei, 1987) based on D values is shown in Fig. 4. Japan Sea and Kanto group formed a single cluster, respectively. However, Pacific Ocean group seemed to consist of three subclusters.

Fig. 4. The genetic relationship among 21 local populations in *Paratya compressa improvisa* drawn by neighbor-joining method. Each population number is identical to that in Fig. 1. Three subclusters in the Pacific Ocean group is surrounded by dotted line, respectively.

4. DISCUSSION

Nei (1975, 1987) noted that heterozygosity in animals was around 0.10, and most invertebrates show an average heterozygosity lower than 0.10. Chow and Fujio (1985a) reported that the heterozygosity values of five palaemonid shrimp species ranged from 0.029 to 0.080. Although, the average heterozygosity of *P. c.*

improvisa populations in present study was only 0.028. This value is small in comparison with other invertebrate species.

Three geographic groups of *P. c. improvisa* were markedly characterized by distributions of predominant alleles at two loci (*Fdp-1* and *Fdp-2*). Genetic distance among the three geographic groups ranged from 0.0572 to 0.2136 with a mean of 0.1281. This value is very large in comparison with those for intraspecific local populations of many other decapod crustaceans (Berglund and Lagercrantz, 1983; Boulton and Knott, 1984; Chow and Fujio, 1985b; Huber, 1985; Nemeath and Tracy, 1979; Tracy et al., 1975), but very similar to that between the American and European lobster species ($D=0.110$) (Hedgecock et al., 1977) and to that between two types of *Palaemon paucidens*. In the case of *P. paucidens*, two D values were reported; one is $D=0.1186$ (Chow et al., 1988) and the other is $D=0.1776$ (Fidhiany et al., 1988). Nei (1975) found that in a variety of animals, D was approximately 1.0 for between-species comparisons, around 0.1 among subspecies, and 0.01 among local races. Ayala (1975) reported that the D value between subspecies, that is, either the first or second step of speciation, is approximately 0.20. According to this rough criterion, genetic distance among the three groups in *P. c. improvisa* falls into the range characteristic of subspecies.

The population structures of freshwater organisms are in general dependent on the distributions of rivers, lakes, and ponds. Species are often divided into subpopulations with varying degrees of gene flow between populations. In the case of *P. c. improvisa*, both significant differences of allele frequencies between populations and random combinations of alleles within each population were found. This indicates that there is very little gene flow among populations. The hierarchical F statistics showed that $N_e \times m$ within each neighborhood were much larger than between neighborhoods. If we assume that each population of *P. c. improvisa* has same N_e (it may be supported by small fluctuation of average heterozygosity among populations), $N_e \times m$ indicates the relative size of migration rate. Thus, we suspect that gene flow within each of three groups is relatively high but those of among groups are low.

Isolation between the Pacific Ocean group and the Japan Sea group has been probably maintained by the barrier of Ohu mountains, which form a backbone in the northern part of Japan. A similar pattern of genetic differentiation has been reported for the Medaka, *Oryzias latipes*, a freshwater fish that inhabits throughout these same regions (Sakaizumi et al., 1983). The basis for the genetic differentiation of the Kanto group from the other groups is unclear at the present time. The three subclusters in the Pacific Ocean group may be due to three water systems, Kitakami, Natori-Hirose, and Abukuma River in this area.

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