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Electrophoretic Evidence of Two Types in the Common Freshwater Shrimp *Paratya compressa compressa* (Decapoda: Atyidae)

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Summary

Fifteen populations of the common freshwater shrimp, *Paratya compressa compressa*, living in the southern part of Japan were examined electrophoretically. Based on the dendrogram constructed from Nei's genetic distance (D) on examination of 19 isozyme loci, these 15 populations were classified into two types (I and II) at a D value of 0.4898. This level of D value is considered to distinguish a subspecies or species. The type I, distributed only in rivers on both sides of the Pacific Ocean and the Sea of Japan, consists of the individuals having *Aat-1*^{b}, *Idhp-2*^{b} and *Ldh*^{c}, while the type II, distributed in lakes, ponds and rivers on the side of the Sea of Japan, consists of the individuals having *Aat-1*^{a}, *Idhp-2*^{a}, *Ldh*^{a} and *Ldh*^{b}. Larger genetic differentiation among populations was evident in the type II ($\bar{D}=0.0920$) including five local populations than the type I ($\bar{D}=0.0027$). Higher gene flow (Nm) among populations was shown in the type I ($Nm=15.375$) than in the type II ($Nm=0.096$). This suggests that the rate of gene flow might depend on the level of expansion in their geographic distributions.

The previous paper (1) revealed that Nei's genetic distance between two *Paratya* subspecies (Decapoda: Atyidae), *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 isozyme loci by starch gel electrophoresis. In further study (2) on the genetic differentiation among 21 populations of *P. c. improvisa*, it has been genetically classified into three geographic groups on the basis of predominating alleles at two fructose-1, 6-diphosphatase loci (*Fdp-1* and *Fdp-2*). However, the genetic differentiation among local populations in the subspecies of *P. c. compressa* has not been analyzed.

Kamita (3) reported the existence of two types (A and B) in the size of eggs in *P. c. compressa*, that is, A-type had larger eggs than the B-type. He also described that A-type is observed in ponds, lakes and rivers, while B-type is

observed in rivers only. Similar phenomenon has been also reported in the other freshwater shrimp *Palaemon paucidence* (4). Two types (A and B) were distinguished by four isozyme loci (*Gpi*, *Mpi*, *Mdh-1* and *Mdh-2*), and the A type was observed in ponds, lakes and rivers, while the B type was only in rivers.

The aim of this study is to demonstrate the existence of two types in *P. c. compressa* on the bases of electrophoretic data, and to examine differences between them in their habitats and morphological characters.

Materials and Methods

Specimens of *Paratya compressa compressa* were collected from 15 different locations in the southern part of Japan during March-August in 1990 to 1993 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 electrophor-

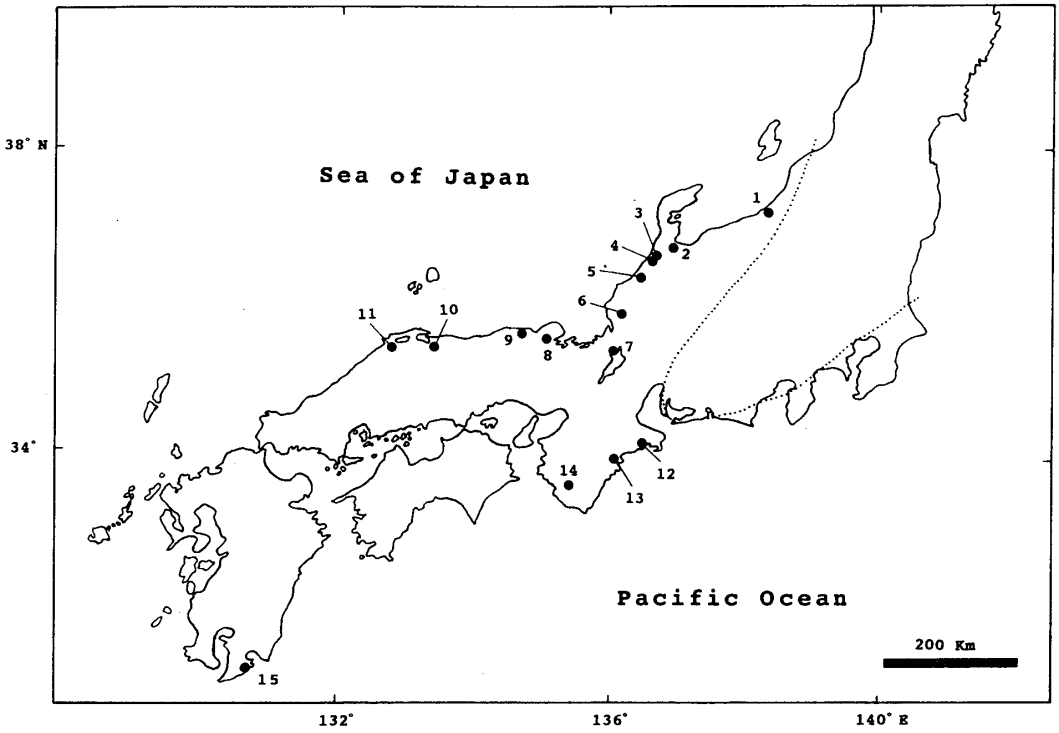

FIG. 1. Map showing collection sites of *Paratya compressa compressa*. Niigata Prefecture: 1=Nagamine Pond; Toyama Prefecture: 2=Pond in Takaoka; Ishikawa Prefecture: 3=Small creek flows into Kahokugata Lake, 4=Kahokugata Lake, 5=Kobagata Lake; Fukui Prefecture: 6=Hino River, Shiga Prefecture: 7=Biwa Lake; Kyoto Prefecture: 8=Pond in Suki; Hyogo Prefecture: 9=Yada River; Tottori Prefecture: 10=Hino River; Shimane Prefecture: 11=Pond in Sanraku; Mie Prefecture: 12=Iseji River, 13=Akaba River; Wakayama Prefecture: 14=Hiki River; Kagoshima Prefecture: 15=Kubota River. A dotted line outlines the geographic range of other subspecies, *P. c. improvisa* descrived by Kamita (1970).

etically analyzed.

The methods of horizontal starch-gel electrophoresis and the designation of alleles and loci have been described in previous papers (1, 2). A locus was assumed to be polymorphic in each local population if the frequency of the most common allele did not exceed 0.95. Average heterozygosity (H_e) were calculated as described by Nei and Roychoudhury (5). Allele frequency data were used to calculatd Nei's genetic distance (D) (6) within and between types. The dendrogram was drawn by using the UPGMA clustering method of Sneath and Sokal (7) based on D values. A coefficient of gene differentiation (G_{ST}) (8) was calculated from allele frequencies. From the G_{ST} values, the rate of gene flow (Nm ; the product of effective size of population and migration rate) in a island model (9) was also estimated. The estimate from G_{ST} was calculated as the formula $Nm = \{(1/G_{ST})-1\}/4$.

Number of rostral spines on the carapace and egg volume were adopted as the morphological character. Ten eggs from each mother shrimp were measured. The egg volume (V) was calculated from the formula $V = \pi LH^2/6$, where L and H are length and width of an egg in mm.

Results

Genetic Differentiation

Allele frequencies at the 19 loci encoding 15 enzymes in 15 populations of *Paratya compressa compressa* are shown in Table 1. Of the loci examined, five loci (*Aldh*, *Alp*, *Fdp-2*, *Est* and *Idhp-2*) were monomorphic in all populations, and ten loci (*Ao*, *Fdp-1*, *G3pdh*, *Gpi*, *Idhp-1*, *Ldh*, *Mdh-2*, *Mpi*, *Pgdh* and *Pgm*) were polymorphic in at least one population. At the other four loci (*Aat-1*, *Aat-2*, *Mdh-1* and *Me*), rare variants segregated in at least one population. In a previous paper (10), we detected two MDH loci (*Mdh-1* and *Mdh-2*) for appearance of two zones of activity in the Biwa Lake population. But all individuals of 14 populations except the Biwa Lake exhibited only *Mdh-1* activity. Thus, we interpreted that the *Mdh-2* of all populations except the Biwa Lake were fixed with a null allele (*Mdh-2°*). At all loci examined, the observed and expected numbers of genotypes were in good agreement with each other.

To estimate the genetic differentiation among populations, the dendrogram was constructed from Nei's genetic distances (D) among 15 populations of *P. c. compressa*. As shown in Fig. 2, two major groups (type I and II) were clearly separated at a D value of 0.4898, to distinguish a subspecies or species level.

Genetic Features in Two Types

A comparison of the allele distribution was made in two major groups (type I and II). Of the loci examined, common alleles between types were not found

TABLE 1. *Allele frequencies at 12 loci in 15 populations in Paratya compressa compressa*

Locus	Allele	Type I					Type II			
		9 (31)	12 (32)	13 (50)	14 (32)	15 (95)	1 (22)	2 (50)	3 (50)	4 (52)
Aat-1	*a*	0.016	0	0	0	0.010	0	0	0	0
	b	0.984	1.000	0.990	0.984	0.980	0	0	0	0
	c	0	0	0.010	0.016	0.010	0	0	0	0
	d	0	0	0	0	0	1.000	1.000	1.000	1.000
Ao	*a*	1.000	1.000	1.000	1.000	1.000	1.000	0	1.000	1.000
	b	0	0	0	0	0	0	1.000	0	0
	c	0	0	0	0	0	0	0	0	0
Fdp-1	*a*	0	0	0	0	0	0	0	0.080	0
	b	0	0	0	0	0	1.000	1.000	0.920	1.000
	c	0.016	0.031	0.070	0.047	0	0	0	0	0
	d	0.984	0.969	0.930	0.953	1.000	0	0	0	0
G3pdh	*a*	0	0	0	0	0.080	0	0	0	0
	b	1.000	1.000	1.000	1.000	0.893	1.000	1.000	1.000	0.981
	c	0	0	0	0	0.027	0	0	0	0.019
Gpi	*a*	0	0	0.010	0	0	0.023	0.200	0.070	0.067
	b	0.145	0.141	0.220	0.188	0.184	0.931	0.090	0.860	0.866
	c	0.839	0.828	0.720	0.781	0.800	0.023	0.070	0.070	0.067
	d	0.016	0.031	0.050	0.031	0.016	0.023	0.620	0	0
	e	0	0	0	0	0	0	0.020	0	0
Idhp-1	*a*	0	0	0	0	0	0	0	0	0
	b	0.226	0.219	0.200	0.344	0	1.000	1.000	1.000	1.000
	c	0.774	0.781	0.800	0.656	1.000	0	0	0	0
Idhp-2	*a*	0	0	0	0	0	1.000	1.000	1.000	1.000
	b	1.000	1.000	1.000	1.000	1.000	0	0	0	0
Ldh	*a*	0	0	0	0	0	0	0.310	0	0
	b	0	0	0	0	0	1.000	0.690	1.000	1.000
	c	1.000	1.000	1.000	1.000	1.000	0	0	0	0
Mdh-2	*a*	0	0	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	0	0
	*o**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mpi	*a*	0.129	0	0	0	0	0	0	0	0
	b	0.742	0.844	0.820	0.781	0.884	0	0	0	0
	c	0.113	0.156	0.150	0.188	0.116	0	0	0	0
	d	0.016	0	0.030	0.031	0	0.909	0.020	0.990	0.981
	e	0	0	0	0	0	0.091	0.940	0.010	0.019
	f	0	0	0	0	0	0	0.040	0	0
Pgdh	*a*	0	0	0	0	0	0	0.090	0.060	0.087
	b	1.000	1.000	1.000	1.000	1.000	0.977	0.910	0.930	0.894
	c	0	0	0	0	0	0.023	0	0.010	0.019
Pgm	*a*	0.016	0.031	0.010	0	0.005	0	0.400	0.020	0.010
	b	0.968	0.953	0.970	0.984	0.995	0.886	0.590	0.980	0.990
	c	0.016	0.016	0.020	0.016	0	0.114	0.010	0	0

Sample size is in parenthesis under each location number (see Fig. 1). *Aldh*, *Alp* and *Est* were monomorphic in all populations, and *Aat-2*, *Fdp-2*, *Mdh-1* and *Me* showed rare variant alleles in at least one population. **o* indicates a null allele (see in text).

TABLE 1. *Continued*

Locus	Alleles	Type II					
		5 (36)	6 (44)	7 (32)	8 (40)	10 (50)	11 (50)
Aat-1	*a*	0	0	0	0	0	0
	b	0	0	0	0	0	0
	c	0	0	0	0	0	0
	d	1.000	1.000	1.000	1.000	1.000	1.000
Ao	*a*	0.903	1.000	0.016	0	0.980	0.770
	b	0.097	0	0.984	0.050	0.020	0.230
	c	0	0	0	0.950	0	0
Fdp-1	*a*	0.111	0	0	0.125	0	0
	b	0.875	1.000	1.000	0.875	1.000	1.000
	c	0.014	0	0	0	0	0
	d	0	0	0	0	0	0
G3pdh	*a*	0.014	0	0	0.050	0	0
	b	0.986	0.966	1.000	0.950	1.000	1.000
	c	0	0.034	0	0	0	0
Gpi	*a*	0.097	0	0.797	0	0.880	0.050
	b	0.792	0.932	0.203	1.000	0.120	0.950
	c	0.111	0.068	0	0	0	0
	d	0	0	0	0	0	0
	e	0	0	0	0	0	0
Idhp-1	*a*	0	0.093	0	0.014	0	0
	b	1.000	0.907	1.000	0.986	1.000	1.000
	c	0	0	0	0	0	0
Idhp-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000
	b	0	0	0	0	0	0
Ldh	*a*	0	0	0	0	0	0
	b	1.000	1.000	1.000	1.000	1.000	1.000
	c	0	0	0	0	0	0
Mdh-2	*a*	0	0	0.469	0	0	0
	b	0	0	0.531	0	0	0
	*o**	1.000	1.000	0	1.000	1.000	1.000
Mpi	*a*	0	0	0	0	0	0
	b	0	0.102	0	0.125	0.010	0.083
	c	0	0.011	0	0	0.990	0.917
	d	0.986	0.807	0.937	0	0	0
	e	0.014	0.080	0	0	0	0
	f	0	0	0.063	0.875	0	0
Pgdh	*a*	0	0	0	0	0	0
	b	0.903	1.000	0.937	1.000	1.000	1.000
	c	0.097	0	0.063	0	0	0
Pgm	*a*	0	0.125	0	0	0.010	0
	b	0.986	0.864	0.969	1.000	0.990	1.000
	c	0.014	0.011	0.031	0	0	0

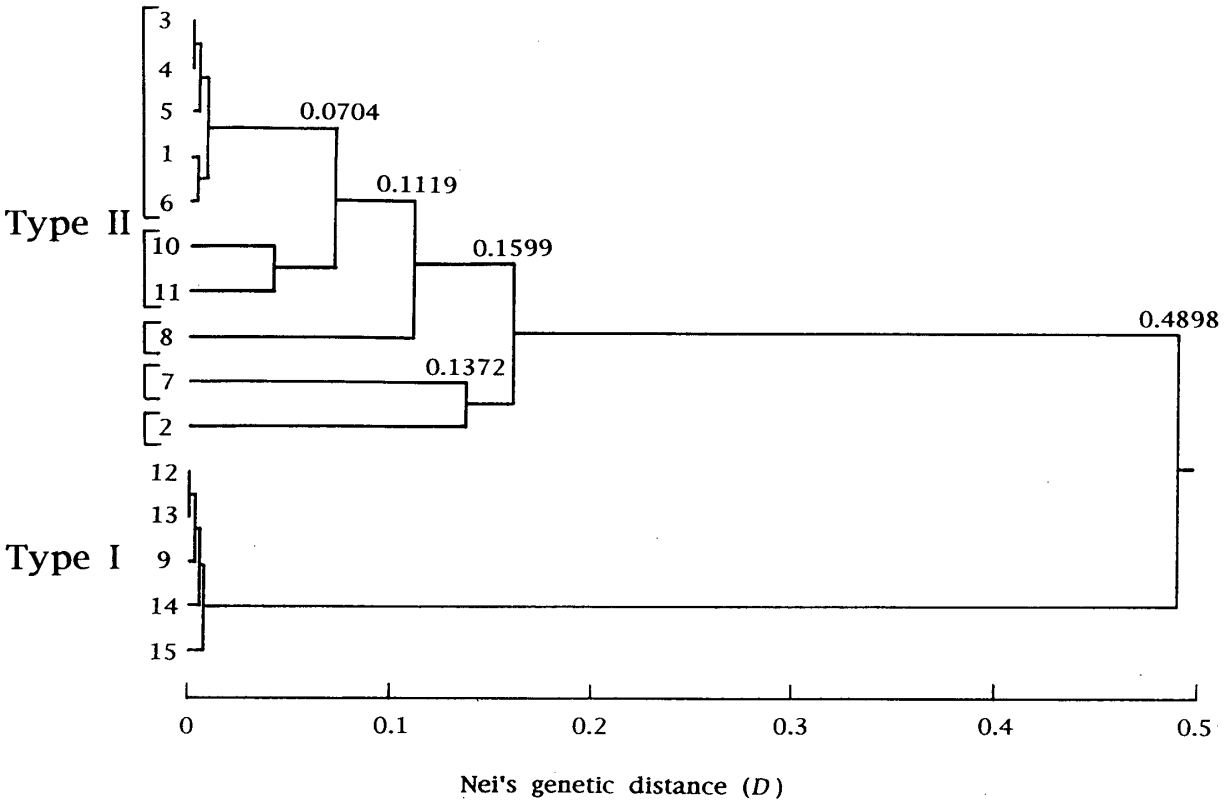

FIG. 2. UPGMA clustering by Nei's genetic distance among 15 populations of *Paratya compressa compressa*. Each population number is identical to that in Fig. 1. Each local population in the type II is showed as a bracket, respectivery.

at three loci (*Aat-1*, *Idhp-2* and *Ldh*), being completely divergent loci. The populations belonging to the type I consisted of individuals having $Aat\text{-}1^b$, $Idhp\text{-}2^b$ and Ldh^c and showed $Aat\text{-}1^a$ and $Aat\text{-}1^c$ genes in moderate frequency. On the other hand, the populations belonging to the type II did not shown $Aat\text{-}1^b$, $Idhp\text{-}2^b$ and Ldh^c genes but consisted of indiviuals having $Aat\text{-}1^d$, $Idhp\text{-}2^a$, and Ldh^b and showed Ldh^a in moderate frequency. At the three loci (*Fdp-1*, *Idhp-1* and *Mpi*), common alleles among the populations belonging to the type II were different from these in type I.

In order to compare between the two types in genetic variability and differentiation, average heterozygosity (H_e), Nei's genetic distance (D), the coefficient of gene differentiation (G_{ST}) and the rate of gene flow (Nm) were calculated (Table 2). The average H_e, there was not significantly different between two types ($t=1.455$, $d.f.=13$, $P>0.05$). Within type I, D values varied from 0.0007 to 0.0077 with a mean of 0.0027. Within type II, D values varied from 0.0004 to 0.1643 with a mean of 0.0920. The diversity level among the populations belonging to the type II was remarkably large but for type I was extremely small. The value of G_{ST} in the type II was about 45 times the value

TABLE 2. *Average heterozygosity ($H_e \pm SD$), Nei's genetic distance (D), coefficient of gene differentiation (G_{ST}) and rate of gene flow (Nm) in two types of Paratya compressa compressa*

	Type I (N=5)	Type II (N=10)
H_e	0.060±0.011 (0.042−0.071)	0.044±0.023 (0.015−0.097)
D	0.0027 (0.0007−0.0077)	0.0920 (0.0004−0.1643)
G_{ST}	0.016	0.722
Nm	15.375	0.096

Range is indicated in each parenthesis.

obtained in the type I. Much higher Nm was evident in the type I than the type II.

From the dendrogram shown in Fig. 2, the type II was consisted with five local populations which were differentiated as subspecies level (0.0704-0.1599).

TABLE 3. *Mean number of rostral spines on the carapace and egg volumes (mm^3) in each population of Paratya compressa compressa*

	Location	Number of rostral spines on the carapace (Mean±SD)	Mean egg volume (mm^3)±SD
Type I	9	2.065±0.250 (31)	–
	12	2.281±0.457 (32)	–
	13	2.360±0.525 (50)	0.019±0.001 (2)
	14	2.469±0.567 (32)	0.024±0.001 (1)
	15	2.316±0.467 (95)	0.014±0.000 (6)
	Average	2.298±0.148	0.019±0.005
Type II	1	1.277±0.429 (22)	–
	2	0.900±0.580 (50)	0.100±0.004 (3)
	3	1.420±0.499 (50)	0.087±0.006 (6)
	4	1.385±0.491 (52)	–
	5	1.222±0.637 (36)	0.087±0.000 (5)
	6	0.955±0.480 (44)	–
	7	1.194±0.543 (32)	0.041±0.001 (2)
	8	1.051±0.686 (40)	0.100±0.001 (6)
	10	0.900±0.544 (50)	0.091±0.005 (6)
	11	0.280±0.454 (50)	0.091±0.002 (3)
	Average	1.053±0.330	0.085±0.020

Locations are given in Fig. 1. Number in parenthesis represents sample size.

Habitats and Morphological Characters

The type I, (locality No. 9 and 12-15) inhabited only rivers on both sides of the Pacific Ocean and Sea of Japan, while the type II (locality No. 1-8, 10 and 11) inhabited lakes, ponds and rivers on the side of the Sea of Japan. The number of rostral spines on the carapace and the egg volume in each population are shown in Table 3. The number of the rostral spines on the carapace varied from 2 to 4 with a mean of 2.298 in the type I, and from 0 to 2 with a mean of 1.053 in the type II. Although the egg volume data was not obtained for all populations, the egg size was larger in the type II (0.041-0.100 mm^3) than the type I (0.014-0.024 mm^3).

Discussion

The existence of two genetically separated types (I and II) and their habitats in *Paratya compressa compressa* in similar to two genetically separated types in *Palaemon paucidence* (4). *P. paucidence* was classified by four loci (*Gpi*, *Mpi*, *Mdh-1* and *Mdh-2*), and the A type was observed in ponds, lakes and rivers, while the B type was in only rivers. Nei's genetic distance (D) between two types in *P. c. compressa* ($D=0.4893$) is similar to the case of *P. c. compressa* and *P. c. improvisa* ($D=0.597$) reported previously by Ikeda *et al* (1).

Data of the habitat and egg size indicate that the types I and II correspond to the B-type (small egg type) and A-type (large egg type) respectively as described by Kamita (3). Individuals having no rostral spine on the carapace which is characteristics to *P. c. improvisa* were observed in some populations and showed large eggs in the type II (A-type). In relation to this phenomenon, Nishino (11) proposed that the A-type of *P. c. compressa* is more closely related to *P. c. improvisa* rather than the B-type of *P. c. compressa*.

From the fact that average heterozygosity was not significantly different between the type I and II, the two types seem to have the same N (the effective size of population). Thus, the rate of gene flow (Nm) within each type is directly comparable. Distinctively larger gene flow and small genetic diversity in the type I than the type II is likely to be attributable to habitat differences. The type I are distributed in only rivers on both sides of the Pacific Ocean and Sea of Japan, while the type II are distributed in lakes, ponds and rivers on the side of the Sea of Japan. Wider distribution and small genetic diversity in the type I might be supported by larger gene flow through the coastal water.

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