Degree of Genetic Differentiation among Different River Populations in Two Estuarine Crabs, Helice tridens and Chiromantes dehaani

Degree of Genetic Differentiation among Different River Populations in Two Estuarine Crabs, *Helice tridens* and *Chiromantes dehaani*

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Summary

Seventeen isozyme loci of the two estuarine crabs, *H. tridens* and *C. dehaani*, inhabiting the same river, were examined by starch gel electrophoresis. To compare the degree of genetic differentiation in *H. tridens* with that in *C. dehaani*, estimates of Nei's genetic distance were determined for the three river populations in the two species. The genetic distance between geographically close river populations was 0.0002 in *H. tridens* and 0.0006 in *C. dehaani*, while the genetic distance between geographically far river populations was 0.0002 in *H. tridens* and 0.0045 in *C. dehaani*. This indicates that the degree of genetic differentiation is remarkably higher in *C. dehaani* than in *H. tridens*. The different levels of gentic differentiation between the wo species could be explained by the different levels of migration rate among river populations.

Isozyme loci are easily detectable by gel electrophoresis and are well known as useful markers for estimating the genetic distance which evaluates the level of genetic differentiation between populations. Previous paper (1) indicated that the genetic distance was 2.775 between the two grapsid crab species, *H. tridens* and *C. dehaani*. The two species are commonly distributed in the estuary of rivers in Japan. Although these species have often been caught together, their main distributions are different from each other in the same river estuary (2, 3, 4, 5). *H. tridens* is distributed mainly in the lower estuary, while *C. dehaani* is distributed mainly in the uppermost of the estuary.

If two populations are isolated from each other geographically, the genetic distance between two populations would be expected to be large. However, migration retards genetic difference considerably ; even a small amount of migration is sufficient to prevent any appreciable genetic difference. Such a situation is primarily responsible for the correlation between geographic distance and genetic distance in the B-type of freshwater shrimp *Palaemon paucidens*, which only inhabit rivers (6). Since the genetic distances were small between neighbor-

ing rivers and lage between geographically isolated rivers, the correlation is expected to be attributed to larval migration.

The aim of the present study is to compare the degree of genetic differentiation in *H. tridens* with that in *C. dehaani* based on isozymic analysis of three different populations.

Materials and Methods

The specimens of *H. tridens* and *C. dehaani* were collected from the Nanakita and Abukuma rivers located in Miyagi prefecture, and from the Shigenobu river in Ehime prefecture. Sampling date, number of individuals in each sample and the carapace width of the specimens are shown in Table 1. To check homogeneity among seasons within the same location, seven collection lots of *H. tridens* from the Nanakita River and two collection lots of *C. dehaani* from the Abukuma River were collected between May and October of 1991 and 1992.

Tissue preparation and procedures for starch gel electrophoresis and nomenclature of isozyme loci followed from the previous paper (1). Statistical analysis for homogeneity among collection lots, and for the degree of genetic difference were the same as previously reported (1).

TABLE 1. *Data of Specimens Surveyed in the Present Study.*

Species Locality	Lot No.	Date of collection		No. of indiv.	Mean C.W.$\pm$Sd (mm)
H. tridens					
Nanakita river	1	2 May	'91	50	28.4$\pm$2.0
	2	3 Jun.	'91	40	26.4$\pm$4.0
	3	3 Jul.	'91	40	28.5$\pm$2.2
	4	5 Aug.	'91	40	28.0$\pm$3.0
	5	10 Sep.	'91	40	25.6$\pm$4.2
	6	16 Oct.	'91	35	28.6$\pm$2.6
	7	8 Jul.	'92	40	27.6$\pm$3.3
Abukuma river	1	9 Jun.	'91	30	18.2$\pm$1.5
C. dahaani					
Nanakita river	1	8 Jul.	'92	40	32.8$\pm$4.2
Abukuma river	1	6 Jun.	'91	30	26.4$\pm$3.2
	2	10 Sep.	'91	35	22.5$\pm$2.8

Results

1. Temporal differences of allele frequencies

The allele frequencies of the 17 loci in the 7 collection lots of *H. tridens* collected from the Nanakita river and in the 2 lots of *C. dehaani* collected from the Abukuma river at different months are shown in Table 2 and 3, respectively. A homogeneity test was performed among lots in each species in order to statistically check the differences of allele frequencies. Notable seasonal fluctuation of allele frequencies of the loci surveyed was not observed in the two species. No significant differences were observed of all loci in each species. Then the pooled allele frequency of each locus in each species was used for the following analyses.

2. Spatial differences among localities in *H. tridens*

The allele frequencies of the 17 loci in *H. tridens* collected from the Nanakita, Abukuma and Shigenobu river are shown in Table 4. The observed number of phenotypes well agreed with the expected number, calculated from allele frequencies under the Hardy-Weinberg equilibrium of all loci in all rivers.

Genetic variability was estimated by number of alleles per locus, proportion of polymorphic loci and average heterozygosity. The number of alleles per locus was 2.0, 1.3 and 1.7 in the Nanakita, Abukuma and Shigenobu river, respecitively ; the proportion of polymorphic loci was 0.000, 0.059 and 0.118, respectively ; and, the average heterozygosity was 0.012, 0.013 and 0.019 in the Nanakita, Abukuma and Shigenobu river, respectively.

In order to clarify the difference of allele frequencies in each locus, t-tests were perfored between every pair of localities. No significant differences were observed of all loci between the Nanakita and Abukuma rivers in Miyagi Prefecture. However, significant differences were observed in the Gpi locus between the Nanakita and Shigenobu rivers, and between the Abukuma and Shigenobu rivers. Nei's genetic distance was 0.0002 between the Nanakita and Abukuma rivers, 0.0001 between the Nanakita and Shigenobu rivers, and 0.0003 between the Abukuma and Shigenobu rivers.

3. Spatial differences among localities in *C. dehaani*

The alleles frequencies of the 17 loci in *C. dehaani* collected from the Nanakita, Abukuma and Shigenobu river and shown in Table 5. Of all the loci examined, the observed number of phenotypes was agreed with the expected numbers of phenotypes. In the Nanakita, Abukuma and Shigenobu rivers, the number of alleles per locus was 1.4, 1.6 and 1.6, respectively ; the proportion of polymorphic loci was 0.180, 0.118 and 0.059, respectively ; and, the average

TABLE 2.   *Allele Frequencies of the 17 Loci in the 7 Lots of*
H. tridens collected from Nanakita River.

| Locus | Allele | lots | | | | | | | Total (285) |
		May '91 (50)*	Jun. '91 (40)	Jul. '91 (40)**	Aug. '91 (40)	Sep. '91 (40)	Oct. '91 (35)	Jul. '92 (40)	
Aat-1	A	0.020	0	0	0	0	0.014	0	0.005
	B	0.970	1.000	1.000	1.000	1.000	0.986	1.000	0.993
	C	0.010	0	0	0	0	0	0	0.002
Aat-2	A	0	0	0	0	0	0	0.013	0.002
	B	0.990	1.000	1.000	1.000	1.000	1.000	0.987	0.996
	C	0.010	0	0	0	0	0	0	0.002
Acp	A	0.090	0.050	0.038	0.013	0.063	0	0.063	0.047
	B	0.910	0.950	0.962	0.987	0.937	1.000	0.937	0.953
αGpd	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gpi	A	0	0	0.013	0.013	0	0	0	0.003
	B	1.000	1.000	0.975	0.987	1.000	1.000	1.000	0.988
	C	0	0	0.012	0	0	0	0	0.009
Idh-1	A	0	0.013	0.012	0	0	0	0	0.004
	B	1.000	0.987	0.988	1.000	0.975	0.986	1.000	0.991
	C	0	0	0.012	0	0.025	0.014	0	0.005
Idh-2	A	0	0.012	0	0	0	0	0.013	0.004
	B	0.990	0.963	1.000	0.975	1.000	0.986	0.975	0.984
	C	0	0.012	0	0	0	0	0	0.002
	D	0.010	0.013	0	0.025	0	0.014	0.012	0.010
Mdh-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2	A	0	0	0	0.013	0	0	0	0.002
	B	1.000	1.000	1.000	0.987	1.000	1.000	0.987	0.996
	C	0	0	0	0	0	0	0.013	0.002
Mpi-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mpi-2	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Odh	A	0	0.013	0	0	0.013	0	0	0.004
	B	1.000	0.987	1.000	1.000	0.987	1.000	1.000	0.996
Pgm	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6-Pgd	A	1.000	1.000	1.000	1.000	1.000	0.986	1.000	0.998
	B	0	0	0	0	0	0.014	0	0.002
Sdh	A	0	0	0	0	0.013	0.014	0	0.005
	B	1.000	1.000	1.000	1.000	0.987	0.986	1.000	0.995
Sod-1	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Sod-2	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

* Number in parentheses represents the number of individuals surveyed.
** for *6-Pgd* the number of individuals surveyed in July '91 is 30, therefore the total is 275.

TABLE 3. *Allele Frequencies of the 17 Loci in the 7 Lots of*
C. dehaani collected from Abukuma River.

Locis Allele		lots		Total (65)
		Jun. '91 (30)*	Sep. '91 (35)	
Aat-1	A	1.000	1.000	1.000
Aat-2	A	0.017	0	0.008
	B	0.983	1.000	0.992
Acp	A	0.067	0.043	0.054
	B	0.933	0.957	0.946
αGpd	A	0.017	0.029	0.023
	B	0.983	0.971	0.977
Gpi	A	0.667	0.600	0.631
	B	0.050	0.043	0.046
	C	0.283	0.357	0.323
Idh-1	A	0.050	0.029	0.038
	B	0.950	0.971	0.962
Idh-2	A	1.000	1.000	1.000
Mdh-1	A	0.983	1.000	0.992
	B	0.017	0	0.008
Mdh-2	A	0	0.014	0.008
	B	1.000	0.986	0.992
Mpi-1	A	1.000	1.000	1.000
Mpi-2	A	1.000	1.000	1.000
Odh**	A	0.260	0.229	0.242
	B	0.740	0.771	0.758
Pgm**	A	0	0.043	0.027
	B	1.000	0.957	0.973
6-Pgd**	A	0	0.014	0.010
	B	1.000	0.986	0.990
Sdh	A	1.000	1.000	1.000
Sod-1	A	1.000	1.000	1.000
Sod-2	A	1.000	1.000	1.000

* Number in parentheses represents number of individuals surveyed.
** the number of individuals surveyed in June 91 are 25 at *Odh*, 20 at *Pgm* and 15 at *6-Pgd*, therefore, the totals are 60 at *Odh*, 55 at *Pgm* and 50 at *6-Pgd*.

TABLE 4. *Allele Frequencies of the 17 Loci in H. tridens collected from Miyagi and Ehime Prefecture.*

Locus	Allele	Nanakita (285)*	Abukuma (30)	Shigenobu (109)**
Aat-1	A	0.005	0	0.005
	B	0.993	0.983	0.981
	C	0.002	0.017	0.014
Aat-2	A	0.002	0.017	0.005
	B	0.996	0.983	0.990
	C	0.002	0	0.005
Acp	A	0.047	0.033	0.050
	B	0.953	0.967	0.950
αGpd	A	1.000	1.000	1.000
Gpi	A	0.003	0	0.014
	B	0.988	1.000	0.945
	C	0.009	0	0.041
Idh-1	A	0.004	0	0
	B	0.991	1.000	1.000
	C	0.005	0	0
Idh-2	A	0.004	0	0
	B	0.984	1.000	0.986
	C	0.002	0	0
	D	0.010	0	0.014
Mdh-1	A	1.000	1.000	1.000
Mdh-2	A	0.002	0	0
	B	0.996	1.000	0.995
	C	0.002	0	0.005
Mpi-1	A	1.000	1.000	1.000
Mpi-2	A	1.000	1.000	1.000
Odh	A	0.004	0.017	0
	B	0.996	0.950	0.995
	C	0	0.033	0.005
Pgm	A	1.000	1.000	1.000
*6-Pgd***	A	0.998	1.000	0.955
	B	0.002	0	0.005
Sdh	A	0.005	0	0.005
	B	0.995	1.000	0.995
Sod-1	A	1.000	1.000	1.000
Sod-2	A	1.000	1.000	1.000

* Number in parentheses represents the number of individual surveyed.
** data for Shigenobu was cited from Irawan *et al.* (1993).
*** for *6-Pgd* the number of individuals surveyed for Nanakita is 275 and 20 for Abukuma.

TABLE 5. *Allele Frequencies of the 17 Loci in C. dehaani collected from Miyagi and Ehime Prefecture.*

Locus Allele		Nanakita (40)*	Abukuma (65)**	Shigenobu (62)***
Aat-1	A	1.000	1.000	0.984
	B	0	0	0.016
Aat-2	A	0.013	0.008	0
	B	0.987	0.992	1.000
Acp	A	0.025	0.054	0.016
	B	0.975	0.946	0.984
αGpd	A	0	0.023	0
	B	1.000	0.977	0.992
	C	0	0	0.008
Gpi	A	0.662	0.631	0.621
	B	0.025	0.046	0.032
	C	0.313	0.323	0.347
Idh-1	A	0	0.038	0.040
	B	1.000	0.962	0.960
Idh-2	A	1.000	1.000	1.000
Mdh-1	A	1.000	0.992	1.000
	B	0	0.008	0
Mdh-2	A	0	0.008	0
	B	1.000	0.992	1.000
Mpi-1	A	1.000	1.000	1.000
Mpi-2	A	1.000	1.000	1.000
Odh	A	0.313	0.242	0.016
	B	0.687	0.758	0.984
Pgm	A	0	0.027	0.016
	B	1.000	0.973	0.976
	C	0	0	0.008
6-Pgd	A	0.013	0.010	0
	B	0.987	0.990	1.000
Sdh	A	1.000	1.000	0.992
	B	0	0	0.008
Sod-1	A	1.000	1.000	1.000
Sod-2	A	1.000	1.000	1.000

* Number in parentheses represents number of individuals surveyed.
** the number of individuals surveyed are 60 at *Odh*, 55 at *Pgm* and 50 at *6-Pgd*.
*** data for Shigenobu river was cited from Irawan *et al.* (1993).

heterozygosity was 0.058, 0.071 and 0.044, respectively.   No remarkable difference was observed among rivers, but genetic variability was higher in *C..dehaani* than in *H. tridens.*

The results of the t-tests for allele frequency between any two rivers showed significant difference at the Odh locus between the Nanakita and Shigenobu rivers, and between the Abukuma and Shigenobu rivers.   A significant difference was not observed among the three rivers of the other loci.   Nei's genetic distance was 0.0006 between the Nanakita and Abukuma rivers, 0.0057 between the Nanakita and Shigenobu rivers and 0.0033 between the Abukuma and Shigenobu rivers. Genetic distance among the three rivers was higher in *C. dehaani* than in *H. tridens.*

Discussion

Seasonal change of allele frequency was not observed in *H. tridens* and *C. dehaani* in the Nanakita and Abukuma rivers.   Irawan *et al.* (1) reported that seasonal change of allele frequencies was not observed in *H. tridens*, *H. japonica* and *C. dehaani* collected from the Shigenobu river in Ehime Prefecture.   Considering their restricted distribution, subdivision of the population was not expected in a single river system in these estuarine crab species.

Between close river populations, no significant difference of allele frequencies was observed ; this was not so between far river populations.   It could be considered that the migration of individuals occurs more frequently between close river population, but less so between far river populations.   Indeed, the larvae of both species could survive and possibly migrate for some period in the sea water after hatching (5) based on salinity tolerance experiments.   Geographical distance between rivers is one of the most important factors of isolation for estuarine species.

However, the level of genetic difference was different between the two species even in the same geographic distance.   Considering that mixing would have to occur in the path through the coastal waters, the most important factor, in adults and in larvae, is the salinity requirement of the species.   Irawan and Kijima (5) indicated a clear difference of salinity requirements in adults and larvae between *H. tridens* and *C. dehaani*.   Adults of *C. dehaani* could survive in fresh-water but adults of *H. tridens* could not.   The larvae of *C. dehaani* could survive in low salinity while *H. tridens* required a salinity of more than 20 ppt.   Also, the adults of *H. tridens* inhabited the lower estuary while *C. dehaani* inhabited the upper-most part of the estuary.   It could be considered that the larvae of *H. tridens* have been able to migrate for long periods and long distances.   Gene migration, then, may have been more widespread in *H. tridens* than in *C. dehaani*.   Therefore, the degree of genetic difference would be lower in *H. tridens*.   In relation to this

observation, Fidhiany *et al.* (7) and Chow *et al.* (8) reported larger genetic differences, between rivers, within the A-type of *Palaemon paucidens* which inhabited upper stream and ponds, than that within the B-type of *P. paucidens*, which inhabited downstream. The different genetic distance observed between *H. tridens* and *C. dehaani* could possibly be explained by the difference in larval migration range in the two species.

Acknowledgements

References

1) Irawan, B., Kijima, A. and Fujio, Y., Genetic divergence among the three species of estuarine crab, *Helice tridens*, *H. japonica* and *Chiromantes dehaani* (Sesarminae, Grapsidae ; Decapoda). *Tohoku J. Agri. Res.*, **43**, 101-110 (1993).

2) Dai, A. and Yang, S., *"Crabs of the China Seas"*, China Ocean Press. Beijing, (1991).

3) Miyake, S., *"Japanese Crustacean Decapods and Stomatopods in Color"*, vol. II, Brachyura, Hoikusha Publishing Co. Ltd., Osaka, (1982). (In Japanese)

4) Sakai, T., *"Crabs of Japan and the Adjacent Seas"*. Kodansha Ltd., Tokyo, (1976).

5) Irawan, B. and Kijima, A., Difference of salinity requirements among the three estuarine crab species *Chiromantes dehaani*, *Helice tridens* and *H. japonica* (Brachyura : Grapsidae). *Tohoku J. Agri. Res.*, **44**, 39-47 (1994).

6) Ikeda, M., Kijima, A. and Fujio, Y., Genetic differentiation among local populations in B-type of *Palaemon paucidens*. *Fish Genet. Breed. Sci.*, **19**, 21-27 (1993). (In Japanese)

7) Fidhiany, L., Kijima, A. and Fujio, Y., Genetic divergence between two types in *Palaemon paucidens*. *Tohoku J. Agri. Res.*, **39**, 39-45 (1988).

8) Chow, S., Fujio, Y. and Nomura, T., Reproductive isolation and distinct population structure in two types of the freshwater shrimp *Palaemon paucidens*. *Evolution*, **42**, 804-813 (1988).