日本産フジノハナガイ科(二枚貝綱)4種のアイソザイム多型と系統の推定

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Isozyme Polymorphism and Inferred Phylogeny in Four Japanese Species of Donacidae (Bivalvia)

Kōji YOKOGAWA¹ and Yasuo KONDO²

Abstract: Isozyme polymorphism of four species of Japanese Donacidae (Bivalvia) (Chion semigranosus, Tentidonax kiusiuensis, Latona cuneata, and Latona faba) were examined by using starch and polyacrylamide gel electrophoresis, and the results of the molecular phylogenetic analysis were compared with the current taxonomic status obtained by morphological analysis of these taxa. Much higher genetic variability than that of the other mollusks was recognized in L. faba and T. kiusiuensis, while that of C. semigranosus and L. cuneata was regarded to be the standard level of the mollusks. The genetic data placed the four species in two clusters, one comprising the two Latona species, and the other consisting of C. semigranosus and T. kiusiuensis. The former is characterized by a roughly triangular shell without a crenulated shell margin, and the latter by a triangular shell with a crenulated shell margin. The molecular phylogenetic analysis corresponds well with the morphological classification.

Keywords: Molecular phylogeny; Donacidae; Isozyme; Latona

Donacid bivalves occur in the intertidal zone of beaches, and are known to show characteristic tidal migration (Mori 1938, 1950; Ansell et al. 1969; Kondo et al. 2001). Some species of Donacidae are commercially exploited in South Africa and Australia. Donax serra is subject to intensive exploitation by bait dealers and anglers in South Africa (de Villers 1975; McLachlan et al. 1996; Laudien et al. 2003). In Australia, Donax deltoides has been collected commercially (Murray-Jones and Steffe 2000).

In Japan, four species of the donacid bivalves, Chion semigranosus, Tentidonax kiusiuensis, Latona cuneata, and Latona faba (Fig. 1) are distributed mainly in the southern regions. Although the donacid bivalves are not commercially exploited in Japan, they are utilized for human consumption in some regions such as Kochi and Okinawa Prefectures. Despite being listed in threatened marine molluscs (Kurozumi 1998), L. cuneata is found to be still common in a wide variety of substrates from very fine sand to gravelly medium sand on beaches of Southwest Japan (Kondo et al. 2001). This is probably because it has finely branched tentacles within an inhalant siphon, to avoid incoming sand grains when feeding. Such wide environmental adaptation of this species may be potentially useful for recreational and commercial exploitation.

In contrast to some recent accumulation of ecological information (Kondo et al. 2001; Takada et al. 2002), there are still some different taxonomic treatments including those between Japanese and overseas researchers, and taxonomy of the donacid bivalves may not be stable. In addition, no genetic information is available by now at least for the donacid bivalves from Japan, by which genetic structures and phylogenetic relation among the four Japanese species can be presumed.

For fisheries science, it is important to grasp the genetic structures of valuable living species as basic information for genetics and breeding technology, and actually much information of...
genetic examinations has been accumulated. While it can also be valuable to evaluate the genetic structures by species systematically, actually Fujio et al. (1983) examined and evaluated isozymic genetic variability of marine molluscs including the other species than the valuable species. Also, Oniwa and Kijima (1989) systematically evaluated interspecific and intergeneric genetic distances (D values) for many marine bivalves. At this standpoint, genetic information of the donacid bivalves is available for such systematic studies in supplementing the basic data.

Therefore, this study performed molecular phylogenetic analysis by using isozyme, which gives general genetic information of the nuclear genome, for the four donacid species occurring in Japan.

Materials and Methods

The sampling data from specimens of the four donacid species (*C. semigranosus*, *T. kiusiuensis*, *L. cuneata*, *L. faba*) used for this study are shown in Table 1. The specimens were preserved in a refrigerator at −80°C for about a month prior to electrophoretic experiment.

The methodology for the genetic examinations is based on Yokogawa (1997), except for the following processes. That is, foot muscle tissue was used instead of adductor muscle tissue in each case, and the digestive diverticulum and foot muscle tissues were homogenized with 40 μl and 80 μl of distilled water, respectively. To detect isozymes in esterase, horizontal thin polyacrylamide gel electrophoresis after Taniguchi and Tashima (1978) was employed, from an extract from the digestive diverticulum tissue.

Enzymes detected by electrophoreses are shown in Table 2, which shows eighteen loci that were common to all the species. Owing to absence of breeding data, the genetic basis of the banding phenotypes on the gels of electrophoresis was assumed by comparison with the homologous enzymes in the reports for the

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**Fig. 1.** General aspects of the four species of Japanese donacid shells. A: *Chion semigranosus* (SL 18.1 mm), B: *Tentidonax kiusiuensis* (SL 7.9 mm), C: *Latona cuneata* (SL 27.5 mm), D: *Latona faba* (SL 21.5 mm).

**Table 1.** Collecting data for specimens examined

<table>
<thead>
<tr>
<th>Species</th>
<th>Japanese name</th>
<th>Date</th>
<th>Locality</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chion semigranosus</em></td>
<td>Fujinohana-gai</td>
<td>Oct. 27, 1996</td>
<td>Ikumi, Toyo, Kochi</td>
<td>4</td>
</tr>
<tr>
<td><em>Tentidonax kiusiuensis</em></td>
<td>Kyusyu-naminoko</td>
<td>Sep. 27, 1996</td>
<td>Okitsu, Kubokawa, Kochi</td>
<td>6</td>
</tr>
<tr>
<td><em>Latona cuneata</em></td>
<td>Naminoko-gai</td>
<td>Sep. 28, 1996</td>
<td>Ikumi, Toyo, Kochi</td>
<td>13</td>
</tr>
<tr>
<td><em>Latona faba</em></td>
<td>Ryukyu-naminoko</td>
<td>Sep. 16, 1996</td>
<td>Iriomote Is., Okinawa</td>
<td>35</td>
</tr>
</tbody>
</table>
other bivalves. The gene nomenclature follows Shaklee et al. (1990), and the alleles were symbolized as relative mobility percentages compared with the most dominant alleles (*100 in the anodal zone, *−100 in the cathodal zone) in *L. faba* at each locus. In addition, capital letters were given to the alleles starting from with the ones nearest to the anodal zone.

Genetic distances (D), following Nei (1972), were calculated with the allelic frequencies of the four species. Also, unbiased values of each D value were calculated following Nei (1990), and 95% confidence intervals based on each unbiased D value were calculated following Shinjo (1986). In addition, a dendrogram of genetic relationships between the species was constructed from the genetic distances by using the unweighted pair group method with arithmetic mean (UPGMA). In the dendrogram, D values were interpreted as years of divergence after methodology of Nei (1990).

**Results**

Values to indicate the genetic features, including average allele numbers per locus, rate of polymorphic loci and average heterozygosity (He, Ho) of the four species are shown in Table 3. Those values of *T. kiusiuensis* and *L. faba* were significantly high, indicating high genetic variability of the two species (Table 3). In particular, it may be notable that *T. kiusiuensis* showed the high values in spite of few specimens (Table 1).

Allelic frequencies at the 18 loci of the four species are shown in Table 4, with some electrophoretograms showing significant isozymes illustrated in Figure 2. Some of these loci and alleles are described as follows:

**Aspartate aminotransferase (AAT):** Although two loci (*AAT-1*, *AAT-2*) were presumed at the AAT, *AAT-1* was not available because it showed weak activities in *C. semigranosus* and *L. cuneata*, and no activity in *T. kiusiuensis*. The *AAT-2* locus clearly appeared in all species (Fig. 2), and was occupied by the *B* (0) allele in *C. semigranosus* and the *A* (45) allele in *T. kiusiuensis*, respectively. In *L. cuneata*, the locus had a major allele of *C* (83) (D) with a frequency of 0.962, and a minor one (*C* 163 (G)). In *L. faba*, the locus had a major allele of *C* (100) (E)
with a frequency of 0.900 and three minor ones of \((*_{-60} [C], *_{-129} [F], *_{-190} [H])\). Namely, the alleles of the four species replaced one another at this locus (Fig. 2; Table 4), further no alleles were shared with the four species.

Glucose-6-phosphate isomerase (GPI): A single locus of GPI was presumed (Fig. 2) and that in each species was polymorphic (Table 4). In \(C.\) semigranosus, the locus had a major allele of \(*_{88} (D)\) with a frequency of 0.875, and a minor one \(*_{31} (H)\). In \(T.\) kiusiuensis, the locus had a major allele of \(*_{131} (A)\) with a frequency of 0.917, and a minor one \(*_{100} (C)\) which was the major allele of \(L.\) cuneata and \(L.\) faba. \(L.\) cuneata and \(L.\) faba had the two common major alleles \(*_{100} (C), *_{65} (E)\), and these two plus several unique minor alleles, respectively.

Leucine aminopeptidase (LAP): A single locus of LAP was presumed and that in each species showed high polymorphism (Table 4). In \(C.\) semigranosus, the locus had a major allele of \(*_{44} (F)\) with a frequency of 0.625, and a minor one \(*_{31} (G)\). In \(T.\) kiusiuensis, the locus had major alleles of \(*_{60} (D)\) and \(*_{52} (E)\) with frequencies of 0.500 and 0.417, respectively, and a minor one \(*_{44} (F)\), which was the major allele of \(C.\) semigranosus and \(L.\) cuneata. In \(L.\) cuneata, the locus included four alleles, and the \(*_{44} (F)\) and \(*_{28} (H)\) alleles which occupied frequencies of 0.423 and 0.346, respectively, were major. In \(L.\) faba, six alleles were detected at the locus, major alleles were the \(*_{100} (A)\) and \(*_{85} (B)\) with a frequency of 0.271 each, indicating very high variability. Among the four species, the \(*_{44} (F)\) allele was common to all the species, the \(*_{60} (D)\) allele was common to \(T.\) kiusiuensis, \(L.\) cuneata and \(L.\) faba, and the \(*_{28} (H)\) allele was common to \(L.\) cuneata and \(L.\) faba (Table 4).

Malate dehydrogenase (MDH): Two loci (MDH-1*, MDH-2*) were presumed at the MDH (Fig. 2), all being monomorphic except for the MDH-1* of \(T.\) kiusiuensis (Table 4). At the MDH-1* locus, it was occupied by the \(*_{390} (B)\) allele in \(C.\) semigranosus, the \(*_{300} (D)\) allele in \(L.\) cuneata, and the \(*_{100} (E)\) allele in \(L.\) faba, respectively. In contrast, \(T.\) kiusiuensis had two major alleles \(*_{460} (A), *_{378} (C)\) at the locus. The MDH-2* locus of \(C.\) semigranosus and \(T.\) kiusiuensis was occupied by the \(*_{145}
Allele particularly has many, the locus comprised five alleles, and the *180 (C) allele included five alleles, and the *180 (C) allele was common to all the species, the *156 (D) allele was common to T. kiusiuensis, L. cuneata and L. faba, and the *144 (E), *100 (F) and *88 (G) alleles were common to L. cuneata and L. faba (Table 4).

Phosphoglucuronate dehydrogenase (PGDH): A single locus for PGDH* was presumed (Fig. 2) and was polymorphic in each species. It was so prominent in L. faba as to have seven alleles (Table 4). The major alleles of the four species differed from one another, that is, the *260 (B) allele in C. semigranosus, the *50 (F) allele in T. kiusiuensis, the *200 (C) allele in L. cuneata, and the *100 (E) allele in L. faba, respectively. Some alleles were common to the four species, such as the *167 (D) allele in C. semigranosus and L. faba, the *260 (B) allele in L. cuneata and L. faba, the *50 (F) allele in T. kiusiuensis and L. cuneata (Table 4).

Phosphoglucomutase (PGM): A single locus of PGM* was presumed and that in each species showed high polymorphism (Table 4). In C. semigranosus, the locus included four alleles, a major allele of *100 (E) occupied a frequency of 0.500, and the *90 (F) allele followed that in the frequency. In T. kiusiuensis, the locus were shared with alleles of *145 (A) and *140 (B)
with a frequency of 0.500 each. In L. cuneata, the locus had a major allele of *100 (E) with a frequency of 0.654, and a minor one (*128 [C]). In L. faba, six alleles were detected at the locus, major alleles were the *100 (E) with a frequency of 0.486, and the *116 (D) and *73 (G) alleles followed that in the frequency. Among the four species, the *128 (C) and *100 (E) alleles were common to C. semigranosus, L. cuneata and L. faba, the *140 (B) allele was common to T. kiusiuensis and L. faba, and the *90 (F) allele was common to C. semigranosus and L. faba (Table 4).

The genetic distances (D values) for the four species, and a dendrogram based on the unbiased genetic distances using the UPGMA method with a scale of divergent year (Nei 1990), are shown in Table 5 and Figure 3, respectively. Regarding these, C. semigranosus and T. kiusiuensis, L. cuneata and L. faba, formed clusters in which genetic distances are about 1.0, respectively. The two clusters were connected at a genetic distance of about 1.4 (Fig. 3).

**Discussion**

In the present study, the numbers of individuals examined of some species might not be sufficient for the genetic analysis. Therefore, accumulation of further isozymic data or additional genetic information such as DNA may be necessary for precise genetic discussion. Although the present study can include such a problem, the results of the present study will be discussed provisionally.

It is well known that genetic variability in the mollusks is extremely higher than that in the other animals (Fujio et al. 1983; Crow 1989). In case of the four donacid species, general genetic variability of C. semigranosus and L. cuneata was regarded to be the standard level of general mollusks, whereas that of T. kiusiuensis and L. faba indicated much higher variability than the other mollusks (Fujio et al. 1983; Crow 1989). In particular, the average heterozygosity (Ho) of L. faba resulted in 0.343, greatly exceeding any values of the 25 mollusks examined by Fujio et al. (1983). Such high genetic variability may be a feature of family Danacidae, otherwise it may be a common feature to marine bivalve shells inhabited tropical regions because striate beach clam, *Atactodea striata* (Mesodesmatidae), which co-inhabits with L. faba in beaches of southern Japan (Kondo et al. 2001), also shows high genetic variability which is match for that of L. faba and T. kiusiuensis (Yokogawa unpublished data).

Oniwa and Kijima (1989) examined and summarized the interspecific and intergeneric genetic distances (D values) for many marine bivalves. In the present study, the D values between C. semigranosus and T. kiusiuensis, and between L. cuneata and L. faba, both are about 0.9 (Table 5), which corresponds well with the specific level in a genus of marine bivalves summarized by Oniwa and Kijima (1989). Here, the D value between C. semigranosus and T. kiusiuensis may be less than the standard generic level of marine bivalves (Oniwa and Kijima 1989), suggesting a phylogenetic closeness of the two genera. While, the D value between the two groups is about 1.4 (Fig. 3), which corresponds to the generic level of marine bivalves (Oniwa and Kijima 1989). This would not support the taxonomic treatment overseas, in which the genus name is unified as *Donax* for the species (Cox et al. 1969; Keen.

Table 5. Genetic distances (D values) between the four donacid species

<table>
<thead>
<tr>
<th>Species</th>
<th>CS*1</th>
<th>TK*1</th>
<th>LC*1</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. semigranosus</td>
<td>0.932</td>
<td>1.186</td>
<td>1.472</td>
</tr>
<tr>
<td>T. kiusiuensis</td>
<td>1.841</td>
<td>1.241</td>
<td>0.885</td>
</tr>
<tr>
<td>L. cuneata</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. faba</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


[Fig. 3](#) Dendrogram based on unbiased genetic distances in four species of Japanese donacid shells. Open bars indicate 95% confidence intervals.
The taxonomic treatment of the four donacid species in Japan is briefly reviewed, as follows: Oyama (1973) regarded the morphological differences in the Japanese donacid species as subgeneric distinctions. Most researchers overseas also treated similar differences as being subgeneric, and commonly used the genus name *Donax* for the species (Cox et al. 1969; Keen 1971; Oliver 1992). In Japan, however, the morphological differences of the donacid species have commonly been regarded as genus level distinctions. For example, Kira (1959) adopted the names *Chion semigranosus* and *Latona cuneata*, and Habe (1961) adopted the names *Chion* (Tentidonax) *kiusiensis* and *Latona faba*. These authors placed the four Japanese donacids into two genera; *Chion* having a triangular shell with the shell margin crenulated, and *Latona* having a more rounded triangular shell without marginal crenulations. Kuroda et al. (1971) and Okutani and Habe (1975) followed these definitions. Subsequently, Habe (1977) elevated *Tentidonax* to the genus, probably owing to its small and elongate shell. He also changed the *Chion semigranosus* to *C. dysoni semigranosus*, regarding it as a subspecies that is distributed in southern Taiwan. Thus, there have been slight changes on the taxonomic status of these donacids, but the general view that the above two groups are valid still holds true. The most recent taxonomic treatment by Matsukuma (2000); however, contradicts our molecular data and most of the previous classification based on shell morphology; *Tentidonax kiusiensis* was placed in the genus *Latona*.

A grouping based on the genetic distances determined for the donacids in this study is consistent with the above-mentioned morphological classification. The smallest genetic distance was inferred for *L. cuneata* and *L. faba* (genus *Latona*), and the second smallest distance inferred for *C. semigranosus* and *T. kiusiensis* (genera *Chion* and *Tentidonax*). These two groups can be phylogenetically independent, respectively.

The general habitats and substrate preferences of *C. semigranosus* and *L. cuneata* are similar to each other, and these species often co-occur on a given beach (Kondo et al. 2001). In addition, complex dendritic structures found near the openings of the inhalant siphons of these species are very similar. However, the inferred phylogeny derived from the isozyme analysis indicates that these two species are the most distantly related among the four species. This suggests that ecological adaptive convergence occurred during the course of their evolution.

The present study revealed the genetic structures of the Japanese donacid bivalves, some of them having extremely higher genetic variability than the other mollusks. Also, it reported their interspecific genetic distances and discussed their correspondence with the taxonomic treatment. Such new information can be useful as additional data for the genetic evaluation of the mollusks systematically. Accumulation of such basic information will necessitate for further studies in the fisheries science.

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**References**


日本産フジノハナガイ科（二枚貝綱）
4種のアイソザイム多型と系統の推定

横川浩治・近藤康生

日本産フジノハナガイ科二枚貝4種, フジノハナガイ Chion semigranosus, キュウシュウナミNokon Tenticidonas kiusiuensis, ナミコガイ Latona cuneata, リュウキュウナミNokon Latona faba のアイソザイム分析によりこれらの遺伝的特徴を調べ, またこれらの系統相関を推定した。T. kiusiuensis と L. faba は遺伝的多様性が著しく大かったが, C. semigranosus と L. cuneata の遺伝的多様性は軟体動物の一般的な水準とみなされた。遺伝的距離は L. cuneata と L. faba の間で最も小さく, 次いで C. semigranosus と T. kiusiuensis の間で小さかった。そしてそれぞれの2種が近縁なグループを形成することが明らかとなった。この結果は, 貝殻の形態に基づく従来の分類を支持している。すなわち, 尖った三角形の殻で, 殻内面に刻みのある Chion と Tenticidonas の両属と, 丸みを帯びた三角形の殻で, 殻内面に刻みのない Latona 属は異なる系統と判断できる。