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**論文 Article**

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# Genetic Relationships among Lancelet Populations in Seto Inland Sea Inferred from Mitochondrial DNA Sequences

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**Abstract:** Genetic relationships were examined among a total of 74 lancelets, *Branchiostoma japonicum* (Willey 1897), collected at 16 stations in the Seto Inland Sea on the basis of the sequence of the *COI* region of mitochondrial DNA. Genetic divergence was usually high at the stations near straits. Besides, there were no significant relationships between geographical and genetic distances of individual lancelets. As many as 62 haplotypes were recognized, among which only three comprised multiple individuals from distant stations, and the remaining ones comprised a single individual. In a dendrogram, some clusters were made up of individuals from nearby stations whereas other ones were made up of those from more or less distant stations. These results suggest that the high genetic heterogeneity of the lancelet population in the Seto Inland Sea is maintained by continuous genetic exchanges via a large-scale dispersion at long planktonic stages driven by tidal and constant currents in this region.

**Keywords:** *Branchiostoma*, genetic relationship, lancelet, population

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## I. Introduction

Lancelets is a member of the subphylum Cephalocordata of the phylum Chordata. They live in relatively coarse sand at the sea bed and spend most of their time in the shallow burrows, filter-feeding small particles, phytoplankton and organic matters (Stokes & Holland 1998). In our previous study, the population of *Branchiostoma japonicum* (Willey 1897), formerly known as *B. belcheri* (Gray 1847), in the Sea of Genkai, situated in the north to the Kyushu Island, Japan, was proven to be genetically closer to that of the Hiroshima Bay in the western Seto Inland Sea than to that of the Osaka Bay in the eastern Seto Inland Sea. This suggested the existence of a barrier to range expansion at the Bisan Strait, situated in the middle of the Seto Inland Sea (Kawai et al. 2003). Besides, a population at a sampling period in the Hiroshima Bay was shown to be genetically distant from that in different sampling periods even at the same site, suggesting the successive settlements of the populations from different origins at an optimal site to their life cycle (Kawai et al. 2006). Furthermore, Saito et al. (2005) examined many *B. belcheri* populations at the different depths and sites, and showed the existence of 4 cohorts at each site and the differences in dominant cohort among the sites, resulting from those in

phytoplankton content. However, the origins and formation processes of the population is still unknown in the Seto Inland Sea.

In this study, we examined the genetic diversity and relationships among the same cohort of the lancelets collected at different sites, covering almost over the Seto Inland Sea, on the basis of the sequence of *COI* region of mitochondrial DNA and discussed some factors determining the genetic structure of the population in the Seto Inland Sea.

## II. Materials and methods

### 1. Samples

Sampling was performed at the 16 stations in the Seto Inland Sea (Fig. 1) from the Toyoshio Maru, a training vessel of Hiroshima University using a Smith-McIntyre grab (0.1 m<sup>2</sup> in sampling area) or a dredge (500 × 200 mm in mouth and 5 mm in mesh) during Feb. '04 to Sept. '06. Lancelets were 'washed out' from the sediment and collected using a sieve (5 mm in mesh) and stored at -20°C.

Lancelet samples were measured for body length and were categorized into cohorts on the basis of the histogram of body length composition (Saito et al. 2005). The cohort of '04 year was used for analysis due to

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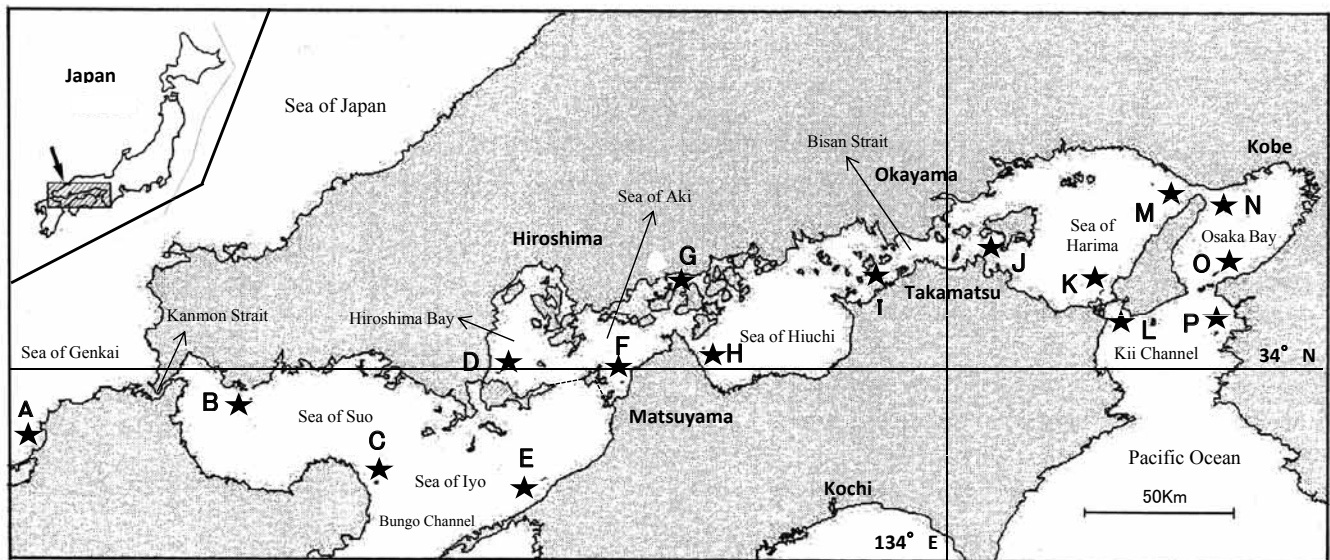


Fig. 1 Map of the 16 sampling stations in the Seto Inland Sea.

availability of the samples at all stations. An individual was used from the cohort for the station H, and three individuals were selected for the station B. Five individuals were selected for other stations. The locations of the sampling stations were shown in Table 1. A total of 74 individuals were examined. Samples were numbered in sequence as A1.

## 2. DNA preparation

Template DNA was prepared from the samples using DNeasy Tissue Kit (Qiagen, Tokyo, Japan), according to the manufacturer's instruction.

Table 1 Location at each station.

	Location	
	Latitude	Longitude
A	33° 44.90'	130° 26.00'
B	33° 53.46'	132° 44.33'
C	33° 36.69'	131° 47.00'
D	33° 58.59'	132° 12.78'
E	33° 36.93'	132° 27.42'
F	33° 56.08'	132° 44.33'
G	34° 19.25'	132° 54.16'
H	34° 06.00'	133° 05.00'
I	34° 20.47'	133° 36.35'
J	34° 24.80'	134° 09.50'
K	34° 15.50'	132° 27.42'
L	34° 11.79'	134° 41.36'
M	34° 38.70'	132° 27.42'
N	34° 34.20'	135° 05.60'
O	34° 21.72'	135° 00.04'
P	34° 13.96'	134° 58.02'

## 3. PCR

COI region (616 bp) of mitochondrial DNA was amplified by PCR with a mixture of a template DNA (50 ng), primers (Nohara et al. 2004: LCO0490, 5'-GGTCAACAAATCATAAAGATATTGG-3'; H6609, 5'-ACTTCAGGGTGACCAAAAAAYCA-3') in a thermal cycler (Mastercycler personal, Eppendorf, Hamburg, Germany) in the following protocol: preheating at 95°C for 2 min. → (denaturation at 95°C for 15 sec. → annealing at 45°C for 15 sec. → extension at 72°C for 30 sec.) × 35 cycles.

## 4. Sequencing

Sequencing was performed directly with a genetic analyzer 3130xl (Applied Biosystem, Calif., USA). Multiple alignment was performed with a ClustalW program (Thompson et al. 1994).

## 5. Evolutionary divergence

Average evolutionary divergence within a station or between the stations were calculated by MEGA (Tamura et al. 2011).

## 6. Dendrogram

Genetic distance between the individuals was calculated by 2-parameter model in Kimura (1980) and a dendrogram was constructed by neighbor-joining method using PAUP 4.0 (Swofford 2000).

### III. Results

#### 1. Evolutionary divergence in each station

Average evolutionary divergence in a station was high at D, G, I, L and N whereas it was low at J and K (Table 2). It was intermediate at other stations.

#### 2. Evolutionary divergence between the stations

Average evolutionary divergence between the stations were shown in Table 3. It was the highest between G and I, N or D, N and I or L, and I and L. It was also high between M or B and I, G, D or L, G and P

Table 2 Estimates of average evolutionary divergence over sequence pairs within stations. The number of base substitutions per site from averaging over all sequence pairs within each station are shown.

Station	Average divergence
F	0.007
M	0.009
B	0.009
I	0.012
C	0.007
G	0.010
K	0.005
N	0.010
O	0.008
D	0.011
P	0.007
L	0.010
E	0.007
J	0.003
A	0.007

or L, and D and I, N or L. It was the lowest between J and K. It was also low between J and F, C, O, P, E or A.

#### 3. Haplotype composition

A total of 62 haplotypes were recognized. Only 3 haplotypes comprised multiple individuals. Among these, a haplotype comprised individuals from the stations C, M and N, another one comprised those from the stations C, K and O, and still another one from the stations A, E, H, J and P. All other haplotypes contained a single individual.

#### 4. Relationship between geographical and genetic distances

There were no significant relationships between geographical and genetic distances of individuals, although genetic distance was inclined to converge to a certain level of *c.* 0.008 between the individuals from far distantly located stations (Fig. 2).

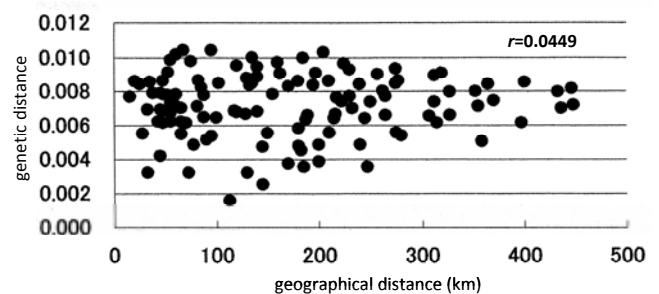


Fig. 2 Relationship between geographical and genetic distances.

Table 3 Estimates of average evolutionary divergence over sequence pairs between stations. The number of base substitutions per site from averaging over all sequence pairs between stations are shown.

	A	B	C	D	E	F	G	I	J	K	L	M	N	O
B	0.008													
C	0.007	0.008												
D	0.009	0.010	0.008											
E	0.007	0.007	0.006	0.008										
F	0.007	0.007	0.007	0.009	0.006									
G	0.009	0.010	0.009	0.011	0.009	0.009								
I	0.009	0.010	0.009	0.010	0.009	0.009	0.011							
J	0.005	0.006	0.005	0.007	0.005	0.005	0.007	0.007						
K	0.006	0.007	0.006	0.008	0.006	0.006	0.008	0.008	0.004					
L	0.009	0.010	0.009	0.010	0.009	0.009	0.010	0.011	0.007	0.008				
M	0.008	0.009	0.007	0.010	0.007	0.008	0.010	0.010	0.006	0.007	0.010			
N	0.009	0.009	0.008	0.010	0.008	0.008	0.011	0.011	0.007	0.008	0.011	0.009		
O	0.007	0.008	0.007	0.009	0.007	0.007	0.009	0.009	0.005	0.006	0.009	0.008	0.009	
P	0.007	0.008	0.007	0.009	0.006	0.006	0.010	0.009	0.005	0.006	0.009	0.007	0.008	0.007

5. Genetic relationships between samples

Largely 3 clusters were recognized; F1, A4-E4-M4-N1 and others. The cluster, A4-E4-M4-N1, was constructed by individuals from far distantly located stations. In the largest cluster, some small clusters, e.g., P1-L4, I5-G4 and J2-L1, were constructed by individuals

from closely located stations while some other small clusters, e.g., D3-M2, D5-N3 and B1-K1, were constructed by those from distantly located ones (Fig. 3). On the other hand, some still other small clusters, e.g., F3-J1, G1-L5 and D4-I4, were constructed by those from moderately distantly located ones.



Fig. 3 Genetic relationships between 74 individuals.

#### IV. Discussion

The most important fact in the present study is a genetic diversity of this organism in the Seto Inland Sea. No less than 62 haplotypes were recognized only in a total of 74 individuals. One explanation to this phenomenon is that a continuous gene flow should extensively occur in this region. Due to inability of positive movement against strong currents at planktonic stages and to residential life of immature stages, there seems to remain a possibility of passive movement by tidal wave or constant currents for large-scale genetic exchange. Indeed, some strong constant currents are known in the Seto Inland Sea (Yanagi & Higuchi 1979; Itami 1984). Kitamura et al. (2005) also explained a high level of diversity in mitochondrial D-loop region of a fiddler crab, *Uca lactea*, at 6 localities in the western Japan by the recruitment of planktonic larvae to a habitat from different origins with coastal currents changing in strength and in direction among years and seasons. Another explanation, although by scarce possibilities, is the constant recruitment of oceanic populations: the Pacific Ocean populations through the Bungo or Kii Channel and the Sea of Genkai population through the Kanmon Strait. This could be partly supported by our previous results on the genetic similarity of the population of the Sea of Genkai to that of the Hiroshima Bay (Kawai et al. 2003).

Evolutionary divergence among the individuals in a station was usually high at the ones situated around the straits. Such genetic diversity is partly attributable to strong tidal currents increasing the chances of recruits from different origins. Evolutionary divergence between the stations were usually high in combinations of the ones across the Bisan Strait (e.g., G or I and N, B or I and L and D, G or I and M). This result supports our previous results based on RAPD analysis (Kawai et al. 2003). Indeed, exchanging movement of sea water in the Seto Inland Sea is known to be interrupted by a sea ridge in this strait (Itami 1984). In contrast, evolutionary divergence was the lowest between J and K in the Harima Nada. It may be explained by the proximity of these stations and a constant current in J to K direction (Yanagi & Higuchi 1979).

On the basis of 2 to 3 months of a long planktonic life in the form of amphioxus larva (Wickstead 1975), a lancelet can move a long distance (more than 1000km)

before settlement (Shanks et al. 2003). Besides, a particular sediment condition is reported to be required for larval settlement of lancelets (Saito et al. 2005). Thus, the recruitments of lancelets of spatially and temporally different origins might be concentrated to an optimal area for settlement. This may be one reason why there were no significant relationships between geographical and genetic distances (See Fig. 2). This is also supported by the seasonal replacements of lancelet populations at a certain site in the Seto Inland Sea in our previous study (Kawai et al. 2006).

In the largest cluster of the dendrogram, some small clusters were constructed by both the individuals from adjacent and distant stations. What is explanation of the mechanism of this phenomenon? The combinations of distant stations comprised those across the Bisan Strait. On the other hand, the population of the Sea of Genkai was proven to be genetically closer to that of the Hiroshima Bay than to that of the Osaka Bay, suggesting the existence of a barrier at the Bisan Strait in our previous study (Kawai et al. 2003). This apparent discrepancy between our results might be partly because an identical cohort to all the stations was examined in this study in contrast to a population comprising multiple cohorts examined in our previous study. A possibility of multiple routes, i.e., via the Bungo Channel or Kanmon Strait and Kii Channel, of the recruitment of a certain oceanic cohort to a habitat in the Seto Inland Sea might be another explanation. A genetically far distantly related individual, F1, to others as well as a cluster, A4-E4-M4-N1, constructed by individuals from far distantly located stations involving an oceanic one A, supports this possibility. Further, juveniles may be possible to move across the shallow straits seeking for better environments. Indeed, Saito et al. (2009) suggested lancelet movements from deep to shallow waters during development seeking for better food environment.

What is the mechanism of establishment of the Seto Inland Sea population? Do some recruitments of oceanic populations occur to the habitats in addition to those of the domestic populations in the Seto Inland Sea. No samples from oceanic populations were examined in this study. On the other hand, Nishikawa (1981) reported the existence of the lancelet populations in the coastal areas of the Pacific Ocean. Further studies involving multiple oceanic populations may lead to the clarification of the

origin to the Seto Inland Sea population and mechanisms for its establishment.

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