Genetic diversity and population structure of a vestimentiferan annelid *Lamellibrachia satsuma* in Japanese and northern Mariana waters

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Received 28 February 2012; Accepted 13 June 2012

**Abstract:** The genetic diversity and population structure of a vestimentiferan annelid, *Lamellibrachia satsuma*, were analyzed based on the nucleotide sequences of mitochondrial DNA obtained from specimens from Kagoshima Bay in Kyushu, Southwestern Japan, which is the shallowest habitat of this species, and Nikko and Daikoku Seamounts in the northern Mariana Arc. The Kagoshima and northern Mariana populations showed significant genetic differentiation, with the genetic diversity of the former being lower than that of the latter. The estimated ages of deviation between the populations in these sea areas as well as expansion of the Kagoshima population suggest the presence of undiscovered habitat(s) of this species in the northwestern Pacific.

**Key words:** genetic diversity, Kagoshima Bay, *Lamellibrachia satsuma*, northern Mariana Arc, population structure

**Introduction**

Chemosynthetic biological communities in which animals depend highly on chemosynthetic primary production by microbes are widely distributed in oceanic environments from shallow waters to trenches and from polar to tropical waters. Determining the distribution and abundance of such communities is important for understanding marine ecosystems. However, they have been rarely investigated in the networks of marine ecosystems probably due to little understanding of connectivity between photosynthetic and chemosynthetic communities as distribution of the latter is limited to reducing environments in each area. Among animals endemic to chemosynthetic biological communities, a vestimentiferan tubeworm, *Lamellibrachia satsuma*, is peculiar because it is distributed in both euphotic and aphotic zones (Hashimoto et al. 1993, Kojima et al. 2001). In addition, it is expected to be used as a model species in studies on the link between chemosynthetic and photosynthetic ecosystems through larval dispersal. Therefore, the estimation of its gene flow and consequent population genetic structure will contribute in understanding the link between the two ecosystems.

*Lamellibrachia satsuma* was described based on the specimens collected from depths of 98–122 m in Kagoshima Bay in Southwestern Japan, which is the shallowest habitat of this species, and Nikko and Daikoku Seamounts in the northern Mariana Arc (Kojima et al. 2001). Recently, additional habitats of this species were discovered in hydrothermal vent fields on Daikoku Seamount (400 m depth) and Dai-ni Kasuga Seamount (460 m depth) in the northern Mariana Arc (Komai et al. 2010).

Kojima et al. (2001) determined the partial nucleotide sequences (624 bp) of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene for 17 *L. satsuma* specimens from Kagoshima Bay and three from the Nankai Trough.
They reported that 16 and 1 specimens from the former and latter sites, respectively, shared identical sequences. The low genetic diversity (gene diversity, 0.12 ± 0.10; nucleotide diversity, 0.00057 ± 0.00065) of the Kagoshima Bay population is also evident from comparisons with congeneric species (Kojima et al. 2001). The habitat in Kagoshima Bay seems to be the shallowest limit of the distributional range of this species. It is situated in the Aira Caldera, which is known to have changed from a freshwater to a marine environment 13,000 years ago (Yamanaka et al. 2010). Because hydrothermalism in Kagoshima Bay is closely linked to the volcanic activity of the Aira Caldera, including Sakurajima Island, the initiation of the present hydrothermal activity is thought to be a relatively recent event. If the population of *L. satsuma* in Kagoshima Bay was recently founded by a few immigrants from outside the bay and/or experienced changes in population size corresponding to the rise and fall of volcanic activity, the low genetic diversity of the population might be attributed to the founder and/or bottleneck effects. Miyake et al. (2006, 2010) hypothesized that the larvae of this species as well as of other species inhabiting the hydrothermal vent field in the northwestern Pacific have been transported from the Japanese waters to the northern Mariana Arc by the Kuroshio Subgyre, which is composed of the Kuroshio Current and Kuroshio Counter Current.

Until recently, no molecular data or specimens for molecular analyses were available for *L. satsuma* from the northern Mariana Arc, except for a single specimen from Nikko Seamount (Black et al. 1997). Therefore it was impossible to evaluate the genetic diversity of the Kagoshima population by comparing with other conspecific populations. In 2005, additional specimens of this species were collected from two hydrothermal vent fields in the northern Mariana Arc, namely, Nikko and Daikoku Seamounts. Although another population of this species was also discovered in the hydrothermal vent fields on Dai-ni Kasuga Seamount, no specimens from this site are available. In the present study, we evaluated the genetic diversity in the Kagoshima population and the genetic structure of this species.

**Materials and Methods**

In total, 22, 20, and 20 *Lamellibrachia satsuma* specimens were collected from Kagoshima Bay (31°39.70′N, 130°48.00′E, 82 m) during Dive #157 of the Remote Operated Vehicle (ROV) *Dolphin* 3K of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Nikko Seamount (23°04.86′N, 142°19.51′E, 456 m) during Dive #496 of the ROV *Hyper-Dolphin* of JAMSTEC, and Daikoku Seamount (21°19.50′N, 144°11.53′E, 399 m) during Dives #491 (N = 18) and 498 (N = 2) of the ROV *Hyper-Dolphin*. All specimens were kept in a freezer at −30°C.

Total DNA was extracted from a small piece (approximately 0.03 cm³) of the vestimentum, which is an anterior muscular part known to be free of endosymbiotic bacteria, by using a DNeasy tissue extraction kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. By using each total DNA as a template, we amplified a part (approximately 1300 bp) of the mitochondrial *COI* gene by using polymerase chain reaction (PCR) with primer sets LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) (Folmer et al. 1994) and COI-6 (5′-GGRTARTCNWRTANCNGNGGGYAT-3′) (Shimayama et al. 1990). The conditions for PCR were 94°C for 60 s followed by 30–40 cycles at 92°C for 40 s, 50°C for 40 s, and 72°C for 90 s. GeneReleaser (BioVenture Inc., Murfreesboro, TN, USA) was used to sequester the products of cell lysis that might have inhibited the polymerase. The nucleotide sequences (1,238 bp) of the amplified fragments were determined using an automated sequencer (ABI3130; Applied Biosystems Inc., CA, USA) with the primers COI-B (5′-GGATGACNGNTAYCCNCCC-3′) (Hasegawa et al. 1996) and HCO2198 (5′-TAAACTTCAGGGTGACCAA-AAAATCA-3′) (Folmer et al. 1994). The nucleotide sequences reported in the present study will appear in the GDDB, DDBJ, EMBL, and NCBI nucleotide sequence databases under the accession numbers AB721479–721496.

A phylogenetic network was constructed on the basis of differences in the nucleotide sequences by the median-joining method using Network version 4.6.1.0 (Bandelt et al. 1999). The genetic diversity of populations was estimated on the basis of both haplotype (gene) diversity (*h*), which is the probability that two randomly chosen haplotypes are different (Nei 1987), and nucleotide diversity (*π*), which is the probability that two randomly chosen homologous nucleotides are different (Tajima 1983, Nei 1987) by using Arlequin version 3.5.1.2 (Excoffier et al. 2010). The differences in the frequencies of haplotypes between populations were examined using the exact test of population differentiation (Raymond & Rousset 1995) performed with Arlequin. The unbiased fixation index, *F*ₜₛ (Weir & Cockerham 1984), was estimated, and its significance was tested using a nonparametric permutation approach (10,000 permutations) performed with Arlequin.

The demographic population history was analyzed based on the distributions of pairwise sequence differences. The probable population expansion was examined by performing mismatch distribution analysis (Rogers & Harpending 1992, Rogers 1995) and by using Arlequin. Whether or not a given population recently experienced a sudden population expansion was determined by testing the null hypothesis that the mismatch distribution expected under the sudden expansion model is different from the observed distribution. If the population was considered to have recently experienced a sudden expansion, the time from the expansion (*t*) was estimated according to the relationship *t* = 2*μu*, where *μ* is the mutation rate per nucleotide and *k* is the number of nucleotides (Rogers & Harpending 1992).
The deviation age between populations was estimated by Bayesian analysis based on coalescence theory (Hey & Wakeley 2001) using the program, IMa (Hey & Nielsen 2007). After performing the analysis with the default settings suggested in the program documentation, the prior distributions of parameters were adjusted. To confirm the results, 10 replicate runs with different seed numbers were performed. For each run, 50 million steps were sampled after a burn-in of 5 million steps.

Results

We identified 18 types of sequences (haplotypes) from 62 individual Lamellibrachia satsuma specimens based on the nucleotide sequences of the mitochondrial COI gene. Of the 1,238 sites, 16 were variable; the transition/transversion bias was 10.63. Identical sequences were obtained from 14 of 22 specimens from Kagoshima Bay. Fifteen of the 22 specimens from Kagoshima Bay were found to be identical based on the same region of the COI gene (624 bp) that was used in the previous study (Kojima et al. 2001). As compared to the haplotypes obtained from Nikko and Daikoku Seamounts, haplotypes from Kagoshima Bay were unevenly distributed in the phylogenetic network (Fig. 1). The genetic diversity of the three populations is shown in Table 1. The diversity of the population from Kagoshima Bay was the lowest among the three populations.

The exact test of population differentiation showed significant genetic differences among the three populations ($p<0.0001$) and between the population from Kagoshima Bay and both the populations from the northern Mariana Arc ($p<0.01$). No significant genetic differences were observed between the two Mariana Arc populations ($p>0.05$). The test performed to examine the unbiased $F_{ST}$ showed a significant genetic difference between the population from Kagoshima Bay and both the populations of the northern Mariana Arc ($p<0.01$). However, no significant genetic differences were observed between the two Mariana Arc populations ($p>0.05$).

Because no genetic deviation was detected between the two populations from the northern Mariana Arc, they were treated as a single population in the subsequent analyses. Mismatch distribution analysis showed that both the population from Kagoshima Bay (mismatch observed mean=2.043, $\tau=5.855$, $\theta_0=0.000$, $\theta_1=2.033$, SSD=0.0421, $p=0.417$) and that from the northern Mariana Arc (mismatch observed mean=3.810, $\tau=6.434$, $\theta_0=0.004$, $\theta_1=10.329$, SSD=0.0201, $p=0.174$) have recently experienced sudden

**Table 1.** The genetic diversity of three Lamellibrachia satsuma populations. Values in parentheses are based on the same region of the COI gene (624 bp) used in the previous study (Kojima et al. 2001).

| Site            | N   | Haplotype | Gene diversity | Nucleotide diversity |
|-----------------|-----|-----------|----------------|----------------------|
| Kagoshima Bay   | 22  | 8 (6)     | $0.60 \pm 0.12$ | $0.00166 \pm 0.00108$ |
|                 |     |           | ($0.54 \pm 0.12$ | ($0.00200 \pm 0.00147$ |
| Nikko Seamount  | 20  | 11 (8)    | $0.91 \pm 0.05$ | $0.00303 \pm 0.00178$ |
|                 |     |           | ($0.84 \pm 0.06$ | ($0.00316 \pm 0.00208$ |
| Daikoku Seamount| 20  | 7 (6)     | $0.83 \pm 0.06$ | $0.00313 \pm 0.00183$ |
|                 |     |           | ($0.82 \pm 0.05$ | ($0.00402 \pm 0.00252$ |

**Fig. 1.** Median-joining network of the haplotypes of Lamellibrachia satsuma. The haplotypes that were not detected in the sample are indicated by squares. The areas of the circles are proportional to the frequency of the occurrence of the haplotypes. The black, gray, and white sectors indicate the relative frequencies of specimens from Kagoshima Bay, Nikko Seamount, and Daikoku Seamount, respectively.

**Fig. 2.** Distribution of the posterior probabilities of deviation age between populations from Kagoshima Bay and the northern Mariana Arc. The results of 10 replicate runs are shown.
expansions in population size. By using the evolutionary rate of vestimentiferan COI genes, namely, 0.23% per million years (Chevaldonné et al. 2002), the expansions of the Kagoshima Bay and northern Mariana Arc populations were estimated to have occurred 1.03 and 1.13 million years ago, respectively.

Figure 2 shows the distribution of the posterior probabilities of the deviation age between the Kagoshima Bay and northern Mariana Arc populations. The range of the mode values of the distributions (i.e., the most probable estimates) and that of the lower limits of the 95% confidence intervals were 2.07–5.02 and 1.02–2.56 million years ago, respectively.

Discussion

The results of the present study show that the population genetic diversity of Lamellibrachia satsuma in Kagoshima Bay was probably underestimated in the previous study (Kojima et al. 2001); however, the genetic diversity is lower than that of the northern Mariana populations (Table 1). Mismatch distribution analysis showed that the Kagoshima Bay population experienced a recent expansion in population size. However, there is little difference in the estimated age of expansion between the Kagoshima Bay and northern Mariana Arc populations. The estimated expansion time of the Kagoshima population is much earlier than when the present volcanic activity of the Aira Caldera started and marine water flowed in the caldera (13,000 years ago; Ishibashi et al. 2008, Yamanaka et al. 2010). The results of coalescent-based analyses showed that the time when the ancestors of the present Kagoshima population deviated from the population in the northern Mariana Arc was much earlier than when the habitat of L. satsuma was founded in Kagoshima Bay (Fig. 2).

Because volcanic activity is a rather unstable phenomenon, the genetic diversity of the Kagoshima population is thought to have been sustained by immigration from other habitats. No habitat of this species has been discovered from other known hydrothermal vent fields in the Japanese waters that are situated in the Okinawa Trough and Izu–Ogasawara Arc areas. Three specimens of this species were collected from a single seep site from the Kanesu-no-se Bank in the Nankai Trough during the 1990s (Kojima et al. 2001). However, no additional specimens have been obtained from this region. Moreover, to date, this species has not been reported from other seep sites in the Nankai Trough even though some of them are situated within the habitable depth range of L. satsuma. Two other vestimentiferan species dominate the seep area of the Kanesu-no-se Bank. Although it is unlikely that the seep areas in the Nankai Trough are one of primary habitats of L. satsuma, the species may have accidentally dispersed to this site and succeeded in settlement.

Based on the discussion mentioned above, we suggest that there are undiscovered habitat(s) of this species in the northwestern Pacific and that the present Kagoshima population was founded by relatively recent immigrants from there. If so, populations of the undiscovered habitats are expected to have almost same genetic characteristics as the Kagoshima population and they are also thought to have deviated from the northern Mariana populations more than 1.02 million years ago. Lamellibrachia satsuma can inhabit both hydrothermal vent fields and cold seep areas. Extensive surveys of deep sea-reducing environments in the northwestern Pacific, especially of unexplored areas such as those around Taiwan and Southeast Asia, should reveal the presently hidden distribution of this species. To understand the formation processes of vent faunas in the Pacific Ocean, it is necessary to evaluate the historical relationships between various species in the northwestern and southern Pacific Oceans.

Acknowledgments

The authors are grateful to the operation teams of the ROVs Dolphin 3K and Hyper-Dolphin as well as to the crew of the support ship Natsushima for help in sampling. Part of this study was conducted as part of the Transcrustal Advection and In-situ bio-geochemical processes of Global sub-seafloor Aquifer (TAIGA) project supported by the Japanese Ministry of Education, Culture, Sports, Science and Technology (Grant No. 20109004).

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