Introduction

The Sea of Japan is a marginal sea surrounded by narrow, shallow straits (Tsushima Strait, 130 m in depth; Tsugaru Strait, 130 m; Soya Strait, 55 m; Mamiya Strait, 15 m). The Sea of Japan became isolated from neighboring waters when the sea level fell during the Pleistocene glacial periods (Ohshima 1990, Tada 1994, Tada & Irino 1999). It has been postulated that the surface water became brackish due to the input of freshwater from the continent (Oba et al. 1991), and anoxic bottom water conditions prevailed during the glacial sea level lowstands (Tada 1994, Tada & Irino 1999). Such environmental changes could have caused the fragmentation and reduction of marine organism populations (Kodama et al. 2008, Kodama & Kojima 2009, Kojima et al. 2001, 2004).

Marine organisms in the Sea of Japan are supposed to possess characteristic intra- or interspecific genetic structures which have been established under the influence of paleoenvironmental changes. To examine the genetic structures of deep-sea organisms in the Sea of Japan using molecular markers would be informative for understanding the effect of environmental changes on the formation of populations there. For this purpose, deep-sea whelks (genera *Buccinum* and *Neptunea* (Buccinidae)) are good target species because direct development is a common reproductive mode in these species (Amio 1963, Golikov 1963, 1980, Webber 1977). Considering their low dispersal abilities, we can expect more detailed information on how paleoenvironmental changes have influence on the genetic structures of marine organisms in the Sea of Japan.

Deep-sea whelks are common in the cold and deep waters of the North Pacific Ocean (Amano & Watanabe 2001). However, several taxonomical problems due to morphological variations and lack of knowledge about gene flows among local populations in deep-sea whelks may lead to inappropriate fisheries management. We and my collaborators examined the genetic inter- and intraspecific relationships of deep-sea *Buccinum* and *Neptunea* species by means of molecular markers with samples from various stations around Japan (mainly from the Sea of Japan). We found some intriguing genetic structures closely related to their characteristic reproductive mode and paleoenvironmental changes in the Sea of Japan. In addition, by comparing inter- and intraspecies relationships of some *Buccinum* species, I clarified some novel taxonomical aspects of these species. Here, I review these genetic structures of deep-sea whelks and describe the future direction of phylogeographical approaches to deep-sea organisms in the Sea of Japan.

**Key words:** *Buccinum*, genetic population structure, molecular phylogeny, *Neptunea*, the Sea of Japan
tant in the deep bottom fisheries (Kato 1979). In particular, *Buccinum bayani* Jousseaume, *B. striatissimum* Sowerby, *B. tenuissimum* Kuroda in Teramachi 1933, *B. tsubai* Kuroda in Teramachi 1933, and *Neptunea intersculpta* (Sowerby) are the representatives of edible whelks in the Sea of Japan (Kato 1979). In Toyama Prefecture, these whelks occupy 80% of fisheries resources of marine mollusks caught by human (Seto 1999). The distinction between *B. striatissimum* and *B. bayani* or between *N. intersculpta* and *N. constricta* is very difficult because their morphologies are highly variable. The difficulty of species identification makes the stock management of the target species difficult because the definition of “species”, which is the basic unit of a reproductively connected population, is essential for the proper stock management of fisheries resources. When the species identification based on morphological characters is difficult, molecular genetic methods offer a promising approach to resolve taxonomical problems.

Here, I review our previous studies on genetic intra- and interspecific relationships of deep-sea whelks around Japan by mainly focusing on the Sea of Japan, and describe several points which should be focused in the next step when targeting these species and the future direction of phylogeographical approaches to deep-sea organisms in the Sea of Japan.

**Genetic population structures of deep-sea *Buccinum* and *Neptunea* species**

The genetic structures of marine organisms are often influenced by their dispersal abilities during the larval stage (Avise 1994). Marine organisms with planktonic larvae are thought to have less genetic structure than those with direct development (Kyle & Boulding 2000). Many deep-sea whelks are known to develop directly into juveniles in their early stages without passing through planktonic stages (Amio 1963, Golikov 1963, 1980, Webber 1977) and are presumed to have a very limited ability to disperse. In case of these direct developing whelks, it is easier to detect genetic differentiation within the species compared to other marine organisms with planktonic larval stage. This also means that not so many recruiters are expected from neighboring localities, and it would be difficult to recover their resources once the stock decreases. Therefore, it is important to examine the degree of genetic connectivity between subpopulations of direct developing whelks in order to define the unit of stock management. In addition, Amano et al. (1997, 2001, 2004) suggested that some deep-sea gastropods became extinct due to environmental changes during the glacial periods in the Sea of Japan. In such a history, to understand how the populations of *Buccinum* and *Neptunea* species are genetically differentiated in the Sea of Japan is an intriguing topic from the view of phylogeogra-

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**Fig. 1.** Schematic view of genetic differentiations of *Buccinum tsubai* and *Neptunea constricta* in the Sea of Japan. *B. tsubai* has four genetically distinct subpopulations whilst the genetic differentiation of *N. constricta* is relatively unclear. Modified with permission from Fig. 2 of Iguchi et al. (2007a). © Springer-Verlag 2006.
phy.

As a first step, we tried to clarify the genetic population structure of *B. tsubai* based on the partial DNA sequences of the mitochondrial 16S rRNA gene by using samples from various stations in the Sea of Japan. *B. tsubai* was chosen because this species is the most common in the Sea of Japan (Kato 1979), and enough samples from various stations were available for the analysis. In addition, this species has a eurybathic distribution pattern (208–1,355 m, Kato 1979). As a result of molecular phylogenetic analysis based on mitochondrial 16S rRNA gene, the haplotypes of *B. tsubai* were grouped into four major lineages (Iguchi et al. 2004). These lineages were clearly patterned geographically in four separate areas characterized by the 1000-m isobath line: the Hokkaido area, the Yamagata–Toyama area, the Yamato Bank area, and the San’in area (Fig. 1). The four areas are clearly separated from each other by complete discontinuity or narrowing of the areas bordered by the 200 and 1,000 m isobaths which almost correspond to the habitat range of *B. tsubai* (208–1,355 m, Kato 1979). This result shows that the subpopulations of each area are almost completely isolated from each other, and the stock management has to be performed by considering each subpopulation as a unit of fisheries resource. Subsequently, we found the morphological differences among subpopulations of *B. tsubai* (Fig. 2; Iguchi et al. 2005). So far, many *Buccinum* species have been described mainly by morphological characteristics (Golikov 1980), but some subpopulations within species may be described as species in the previous taxonomy. The variation of morphological characters shown among subpopulations of *B. tsubai* would help the inference of the range of morphological variation within *Buccinum* species.

We also performed the comparative analysis on the genetic population structures of two deep-sea whelks *Buccinum tsubai* and *Neptunea constricta* in the Sea of Japan by using the partial sequence of mitochondrial cytochrome oxidase subunit I (COI) gene. COI gene is known to be highly variable and more informative for population analysis than 16S rRNA gene (Hart and Podolsky 2005). In our results, *B. tsubai* included four genetically distinguishable subpopulations, which is consistent with the result using mitochondrial 16S rRNA sequences (Iguchi et al. 2004, 2007a). The genetic structure of *N. constricta* was also clear and significant genetic differentiation was detected by analysis of molecular variance (AMOVA) among the four regions corresponding to the distribution of *B. tsubai* subpopulations, but the degree of the genetic differentiation was lower than that of *B. tsubai* (Fig. 1, Iguchi et al. 2007a). The difference between the genetic structures of these two species could be explained by two possibilities, namely, a difference in either their dispersal abilities or the historic

![Hokkaido](image1.png)

![Yamagata-Toyama](image2.png)

![San’in](image3.png)

![Yamato Bank](image4.png)

**Fig. 2.** Morphological variations of *Buccinum tsubai* in the four subpopulations from the Sea of Japan. Scale bar = 1 cm.
distribution patterns of these species. If *N. constricta* has higher dispersal ability than *B. tsubai*, the difference of genetic structures of these two species would occur. However, the habitat range of *N. constricta* is almost the same as that of *B. tsubai* (250–1,350 m, Kato 1979). In addition, *N. constricta* is also thought to be a direct development species (Iguchi et al. 2007a). Therefore, the dispersal abilities of these species would be similar to each other. A fossil of *B. tsubai* was recorded from the Pliocene on the Sea of Japan side, whereas no *N. constricta* fossil has been reported there (Amano 1997, Amano & Watanabe 2001), which suggests another possibility that *B. tsubai* has already existed before *N. constricta* entered into the Sea of Japan. The nested clade analysis of mitochondrial COI sequences of *N. constricta* suggested the recent expansion of the distribution of this species (Iguchi et al. 2007a). The difference between the genetic population structures of these two species would be attributed to the historic distribution patterns of these species. But our analysis was based on a small number of samples, and reanalysis with more samples should be performed in the future to confirm the idea above.

Based on mitochondrial COI sequences of *B. tsubai*, we calculated genetic diversity of each subpopulation of this species, and found that the value of the Yamato Bank subpopulation is extremely low. This would be caused by genetic drift due to the small population size because the habitat range of the Yamato Bank subpopulation is smaller than those of other subpopulations. In addition, our sampling by 19 sweeps of the trawl could collect only 14 samples of this species in the Yamato Bank although several hundred samples could be available in other subpopulations. These suggest the smaller population size of the Yamato Bank subpopulation of *B. tsubai*. The divergence time among four subpopulations of *B. tsubai* was inferred as 0.42–1.46 million years ago with the dominant haplotypes of each subpopulation based on a nucleotide divergence rate of 2.4% per million years (Hellberg & Vaucquer 1999). This large divergence time suggests that *B. tsubai* could survive in the Sea of Japan through some glacial periods despite the deterioration of its habitat. *B. tsubai*'s survival in the Sea of Japan during the glacial period might be attributed to the existence of intermediate seawater maintaining normal salinity and oxygen concentration which make it possible for *B. tsubai* to survive between the low-salinity surface water and anoxic deep water during the glacial periods. The possibility of the existence of this intermediate seawater is suggested by radiolarian fossils, and this intermediate water is inferred to have prevailed at depths ranging from 200 to 400 m during the glacial periods in the Sea of Japan (Itaki 2001, Itaki et al. 2004). Considering the habitat depth of *B. tsubai* (208–1,355 m, Kato 1979), it is thought that this species could survive at the intermediate seawater during the glacial periods in the Sea of Japan (Amano 2004). The reduction of the habitat range of *B. tsubai* during the glacial periods in the Sea of Japan would also have facilitated the genetic differentiation among subpopulations of this species. This tendency is clear especially in the Yamato Bank population which is well recognized in the larger branch length of genotypes from the Yamato Bank subpopulation in the molecular phylogenetic trees (Iguchi et al. 2004, 2007a).

The monophyly of the San’in subpopulation and that of the other three subpopulations were supported by phylogenetic analysis (Iguchi et al. 2004, 2007a) contrary to our expectation that the monophyly of the San’in subpopulation and that of Yamato Bank one would be supported considering these geographic positions. This means that the San’in subpopulation was the first to be separated from the other three subpopulations in the past. A very deep bottom (the Yamato Basin) separates the Yamato Bank subpopulation and the Yamagata–Toyama subpopulation, and it is difficult for individuals of the Yamato Bank subpopulation to cross this deep bottom and exchange with those of the Yamagata–Toyama subpopulation. Thus, individuals of the Yamato Bank subpopulation are thought to have passed through the area occupied by the present San’in subpopulation to exchange with those of the Yamagata–Toyama subpopulation. However, the Yamato Bank subpopulation and the San’in subpopulation are not monophyletic. The present San’in subpopulation might originally have come from habitats near the continental China or the Korean Peninsula considering the present location. Further analysis of populations near the continent should be performed to elucidate the reliable phylogenetic relationship among subpopulations of *B. tsubai*.

**Molecular phylogeny for Buccinum species**

About the taxonomy of *Buccinum* species based on morphological characters, serious taxonomical confusion remains (Amano & Watanabe 2001). For example, Golikov (1980) described 86 species and six subspecies globally. On the other hand, Tiba and Kosuge (1984) described 107 species and nine subspecies only from the North Pacific, and Higo et al. (1999) 68 species and 13 subspecies around Japan. These differences in numbers of species and subspecies well reflect the wide range of morphological variations of *Buccinum* species (Amano & Watanabe 2001). As described above, *Buccinum* species perform direct-development and their dispersal abilities seem to be very low (Amio 1963, Golikov 1980, Webber 1977, Seto 1999, Illamo et al. 2003). Therefore, some local populations within a single species may have been described as different species or subspecies. Molecular genetic approaches would provide novel insights into these taxonomical problems in these gastropods, however, few genetic studies on this taxon have been reported (Harasewy et al. 1997, Hayashi 2005, Shirai et al. 2010).

We performed molecular phylogenetic analysis using partial nucleotide sequences of a mitochondrial 16S rRNA gene in eight *Buccinum* species: *B. aniwamun* (Dall, 1907); *B. bayani* Jousseaume, 1883; *B. tenuissimum* Kuroda in
Teramachi, 1933; *B. striatissimum* Sowerby, 1899; *B. miyauchii* Azuma, 1972; *B. inclytum* Pilsbry, 1904; *B. isaotakii* Kira, 1959; and *B. tsubai* Kuroda in Teramachi, 1933, which include four genetically different subpopulations (Iguchi et al. 2004, 2007a) as described above. In this analysis, we added the genetic data of four subpopulations of *B. tsubai* in order to compare the genetic distances between interspecies and intraspecies. On the molecular phylogenetic tree based on mitochondrial 16S rRNA gene, the haplotypes were grouped into four major clades (Fig. 3, Iguchi et al. 2007b). The species belonging to the same clade shared similar morphological characters on shell and penis. Considering the genetic distances, sympatric distribution, and the morphological differences among clades, the species among clades would be reproductively isolated. But the phylogenetic relationship among these clades was unclear and more species should be added in the future analysis.

The average genetic distances among the four clades were significantly larger than those within each clade (Fig. 3, Iguchi et al. 2007b). On the other hand, the average genetic distances within two clades, namely, the clade including *B. tsubai* subpopulations (Tsubai group) and that including four *Buccinum* species sharing similar shell shape (*B. aniwanum*, *B. bayani*, *B. striatissimum*, *B. tenuissimum*: Aniwabai group), were almost the same (Iguchi et al. 2007b). Considering no overlapping of geographic distribution of the subpopulations of *B. tsubai* and the morphological differences among the subpopulations (Fig. 2, Iguchi et al. 2005), these subpopulations may correspond to subspecies. Considering that the average genetic distance within the clade of Aniwabai group is almost equal to that of Tsubai group, the Aniwabai group may include some subspecies. However, their species relationship is somewhat difficult to interpret. About *B. striatissimum* and *B. tenuissimum*, we previously suggested that these two species are likely to be different species because they show sympatric distribution and are genetically isolated (Iguchi et al. 2008). However, recent analysis with large number of samples showed that hybridization may be occurring between these two species (Shirai et al. 2010). The reason why *B. bayani* and *B. tenuissimum* shared the same haplotype is also unclear. Analysis by other molecular markers (faster evolving genes and nuclear DNA) would be necessary in the future.

**Concluding remarks**

At present, the information on the genetic structures of marine organisms in the Sea of Japan is accumulating (Kodama et al. 2008, Kodama & Kojima 2009, Kojima et al. 2008).
Amano K (1997) Biogeography of the genus Buccinum species should be considered by using enough samples per species. For species delimitation of Buccinum species, sympathy and genetic distances based on some molecular markers including nuclear DNA should be considered by using enough samples per species.

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