

論文の内容の要旨

論文題目 Phylogeographic study of the Asian horn snail

Batillaria attramentaria (Mollusca, Gastropoda)

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The Asian horn snail *Batillaria attramentaria*, is an intertidal gastropod which predominates in Japanese coastal areas. In the previous study based on the nucleotide sequences (384 base pair (bp)) of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene, individuals of *B. attramentaria* were divided into two phylogenetically distinct groups, which are distributed along two warm currents. Such a genetic structure of *B. attramentaria* was attributed to its low dispersal ability which relates to the direct development. The spatial patterns of genetic variation were considered to reflect the historical environmental changes. On the other hand, *B. attramentaria* was unintentionally transported with oyster seedlings to the North America in the early 1900s and has displaced native confamilial mudsnails through competition for food sources. In recent years, the artificial transportation has also been reported within Japan. Therefore, the present distribution of *B. attramentaria* is thought to have been affected by both historical major environmental changes such as the glacial-interglacial cycle and the human-mediated transport. Revealing the detailed population genetic structure and gene flow between local populations of *B. attramentaria* is helpful to reveal the influence of the past environmental changes on organisms in the tideland and to reconstruct histories of local populations.

A detailed population structure of *B. attramentaria* and historical process of its formation were analyzed based on longer sequence (1020 bp) of the COI gene than the previous study in Chapter 2. The 918 individuals of *Batillaria attramentaria* sampled from 47 local populations yielded 81 haplotypes. These were divided into two phylogenetically

distinct groups which are distributed along two warm currents (the Kuroshio group and the Tsushima group) as the previous study. Although all haplotypes of the Kuroshio group could be divided into 5 subgroups, no clear genetic structure was recognized in the Tsushima group. No genetic difference was detected between most local populations which dominated by individuals of same subgroups. The population history of *B. attramentaria* was reconstructed by the coalescent-theory based analyses, and their genetic structure was suggested to have been formed during the interglacial–glacial cycle in the Quaternary. The human-mediated introduction was suggested by discontinuous distributions of some haplotypes in the Tokyo Bay and the Tohoku district.

It is difficult to determine whether genetic characteristics of each local population are a result of the historical events or the recent anthropogenic introduction. More informative genetic markers were needed to detect a recent gene flow between local populations which leaves no trace in nucleotide sequences of the COI gene. Polymorphic microsatellite loci are suitable for determining more detailed population genetic structure and gene flow than a single mitochondrial gene. In Chapter 3, 14 microsatellite markers for *B. attramentaria* were developed using two discrete techniques, the dual-suppression polymerase chain reaction (PCR) technique and next generation DNA sequencing. Additionally, 2 multiplex panels were designed for cost and time reduction. These microsatellite markers and the high-throughput methodology using multiplex PCR panels are expected to provide useful tools for assessing the population genetic structure, revealing gene flow among local populations, and detecting whether interbreeding occurred between transported individuals and natives.

Detailed population genetic structures and contemporary gene flow among local populations of *B. attramentaria* were revealed by using 14 microsatellite markers. The results of the Bayesian clustering approach suggested that local populations of *B. attramentaria* around Japan were divided into 3 clusters, which were mainly distributed in (1) areas around the northern Kyushu Island and Korea and the Japan Sea (East Sea) coasts of Honshu Island, (2) the Sea of Okhotsk and Pacific coasts of Hokkaido Island and the Sanriku Coast and (3) the Pacific coast from the Kanto to Kyushu districts. Discrepancy between results based on two genetic markers might be attributable to difference in their resolution power. In spite of the low dispersal ability, the significant migration between local populations, probably using ocean currents, via floating in juvenile periods or rafting on drifting seaweeds, was suggested. On the other hand, the presence of local populations at least a part of which had been formed by the human-mediated introduction was suggested in Tokyo Bay, the Tohoku district, and around Hokkaido Island.

The comprehensive examination of results by both genetic markers suggested that the local population in Ouchimura Lake, Aomori Prefecture were founded by recent immigrants due to the unintentional transport associated with release of short-necked clams transported from Aichi Prefecture and that of Miyako Bay, Iwate Prefecture may be formed by transported and domestic individuals as well as those of mixed race. In Tokyo Bay, the local population in Yatsu Tideland was suggested to have founded recently by immigrants from

Obitsugawa Estuary where the unintentional introduction from Ariake Bay, the Seto Inland Sea, and the Tohoku district was also suggested.

By using two distinct genetic markers which are different in the hereditary mode and the resolution, the detailed phylogeographic structure, histories of local populations, and connectivity among local populations were revealed for *B. attramentaria*. The present genetic structure of this species was shown to have been formed by not only historical environmental fluctuations but also contemporary human activities. The present study will greatly contribute to understand the influences of environmental changes and human activities on coastal species around Japan.