DOCTORAL THESIS

Population connectivity, local adaptation, and biomineralization of deep-sea mussels (Bivalvia: Mytilidae) in Northwestern Pacific

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ABSTRACT

The discovery of deep-sea chemosynthesis-based ecosystems including hydrothermal vents and cold seeps has greatly expanded our view of life on Earth. Nevertheless, for many benthic organisms in these ecosystems, little is known about where they come from, how scattered populations are connected by larval dispersal, and how they adapt to the local environments. Mussels of *Bathymodiolus platifrons* (Bivalvia: Mytilidae) are one of the dominant and foundation species in deep-sea chemosynthesis-based ecosystems. They are known to have a wide geographic distribution, and are also one of the few deep-sea species capable of living in both hydrothermal vents [in Okinawa Trough (OT)] and methane seeps [in the South China Sea (SCS) and Sagami Bay (SB)]. Previous population genetics studies of *B. platifrons* mostly relied on one to several genes, which suffered from the lack of sensitivity required to resolve their fine-scale genetic structure, and were unable to reveal their adaptation to the local environments. With the repaid development of molecular techniques, it is now possible to address their demographic mechanisms and local adaptation from a genome-wide perspective.

Therefore, in the first part of my thesis, I aimed to generate genome-wide single nucleotide polymorphisms (SNPs) for *B. platifrons* via a combination of genome survey sequencing and the type IIB endonuclease restriction-site associated DNA (2b-RAD) approach, assess the potential use of SNPs in detecting fine-scale population genetic structure and signatures of diversifying selection, as well as their cross-species application in other bathymodioline mussels. Genome survey sequencing was conducted for one individual of *B. platifrons*. *De novo* assembly resulted in 781 720 sequences with a scaffold N50 of 2.9 kb. Using these sequences as a reference, 9307 genome-wide SNPs were identified from 28 *B. platifrons* individuals collected from a methane seep in the SCS and a hydrothermal vent in the middle OT (M-OT), with nine outlier SNPs showed significant evidence of diversifying selection. The small F_{ST} value (0.0126) estimated based on the neutral SNPs indicated high genetic connectivity between the two populations. However, the permutation test detected significant differences ($P < 0.00001$), indicating the two populations having clearly detectable genetic differentiation. The Bayesian clustering analyses and principle component analyses (PCA) performed based on either the neutral or outlier SNPs also showed that the two populations were genetically differentiated. This initial study successfully demonstrated the applicability of combining genome sequencing and 2b-RAD in population genomics studies of *B. platifrons*. Besides, using the survey genome of *B. platifrons* as a reference, a total of 10 199, 6429, and 3811 single

nucleotide variants (SNVs) were detected from three bathymodioline mussels *Bathymodiolus japonicus*, *Bathymodiolus aduloides*, and *Idas* sp. These results highlighted the potential of cross-species and cross-genus applications of the *B. platifrons* genome for SNV/SNP identification among different bathymodioline lineages, which can be further used in various evolutionary and genetic studies.

To have a deeper understanding of how individuals of *B. platifrons* are connected among and adapt to their habitats, in the second part of my thesis, I used both mitochondrial genes and genome-wide SNPs to conduct a more comprehensive population genetics/genomics study of *B. platifrons*. Three mitochondrial genes (i.e. *atp6*, *cox1*, and *nad4*) and 6398 SNPs generated by 2b-RAD were obtained from 110 *B. platifrons* individuals from six representative locations along their known distribution range in the Northwestern Pacific. The small F_{ST} values based on both types of genetic markers all revealed high genetic connectivity of *B. platifrons*, which may have been driven by the strong ocean currents (i.e. Kuroshio Current, North Pacific Intermediate Water). However, when using SNP datasets rather than mitochondrial genes, individuals in the SCS were identified as a distinct genetic group, indicating the Luzon Strait may serve as a dispersal barrier that limits their larval exchange between the SCS and the open area in the Northwestern Pacific. Moreover, a genetic subdivision of *B. platifrons* in the southern OT (S-OT) from those in M-OT and SB was observed when using 125 outlier SNPs for data analyses. The outlier-associated proteins were found to be involved in various biological processes, such as DNA and protein metabolism, transcription and translation, and response to stimulus, indicating local adaptation of *B. platifrons* even they are confronted with extensive gene flow in the OT-SB region. Furthermore, by using SNP datasets, populations in S-OT were revealed to be the source of gene flow to those in the SCS, M-OT, and SB. Overall, these results offered novel perspectives on the potential forces that may have led to the genetic differentiation and local adaptation of *B. platifrons*, which can serve as an example for other deep-sea species with high dispersal potential, and contribute to the designation of marine protected areas and conservation of deep-sea chemosynthesis-based ecosystems.

Molluscan shell formation is one of the most common and abundant biomineralization processes in metazoans. Although composed of less than 5 wt% of the molluscan shells, shell matrix proteins (SMPs) are known to play multiple key roles during shell formation, such as providing a gel-like micro-environment to favour mineral precipitation, promoting crystal nucleation, as well as guiding and inhibiting crystal growth. To date, all studies on SMPs have focused on molluscs in terrestrial and shallow-water ecosystems with no reports for those living in the deep ocean.

Herein, the third part of my thesis was to study the shell proteomes of *B. platifrons* and its shallow-water relative *Modiolus philippinarum* with the aim to bridge such knowledge gaps in biomineralization studies. A total of 94 and 55 SMPs were identified from the shell matrices of *B. platifrons* and *M. philippinarum*, respectively, with 31 SMPs shared between two species. These SMPs can be assigned into six broad categories, comprising calcium binding, polysaccharide interaction, enzyme, extracellular matrix-related proteins, immunity-related proteins, and those with uncharacterized functions. Many of them, such as tyrosinases, carbonic anhydrases, collagens, chitin-related proteins, peroxidases, as well as proteinase and proteinase inhibitor domain-containing proteins, have been widely found in molluscan shell matrices and other metazoan calcified tissues (e.g. exoskeletons of corals, tubes of tubeworms), whereas some others, such as cystatins, were found for the first time in molluscan shell matrices, and ferric-chelate reductase-like proteins and heme-binding proteins were to be detected for the first time in metazoan calcified tissues. This is the first report of the shell proteome of deep-sea molluscs, which will support various follow-up studies to better understand the functions of these SMPs, especially in relation to environmental adaptation.

Overall, my population genetics/genomics studies have improved our understanding of the population dynamics, genetic connectivity, fine-scale genetic structure, and local adaptation of *B. platifrons* in the Northwestern Pacific, and my proteomics study has shed light on the biomineralization processes of molluscs in the deep ocean.

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