

Were marine microplankton in the Japan Sea geographically isolated during the Last Glacial Maximum?

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The Japan Sea is connected by three straights (the Soya, Tsugaru, and Tsushima Straights) to the Sea of Okhotsk and Pacific Ocean with the shallow sill depth (140 m). During the last glacial maximum (LGM: 23-19 kilo years before present), the sea-level was decreased at least 120 m lower than today and the Japan Sea was almost isolated from surrounding seas. It is possible that such geographic isolation reduced and/or impeded gene flow of marine organisms between the Japan Sea and surrounding seas. Previous phylogeographic studies of coastal vertebrates (only whose larval stage is planktonic) have actually suggested that the Japan Sea was closed during the LGM. However, there is no phylogeographic study with marine microplankton, which inhabit the water column throughout their entire life cycle.

Radiolaria, the major marine planktonic protists, are passively transported in the pelagic ocean. Their geographic distribution would be easily affected by geographic changes through geological time. Moreover, their siliceous shells have been preserved in marine sediments and form a good fossil record. The sensitivity to geographic changes and well-preserved fossil record of Radiolaria could allow us to elucidate a past geographic isolation of marine microplankton. *Larcopyle buetschlii*, a morphospecies of radiolarians analyzed in the present study, is found in the surface waters in the Pacific Ocean, whereas it has a characteristic distribution vertically ranged from the surface to deep layers in the Japan Sea. In addition, its fossil specimens are continuously observed in the Japan Sea before the LGM. Therefore, *L. buetschlii* could be a good model to study a link between geographic isolation during the LGM and reproductive isolation of marine microplankton.

Heterogeneity of internal transcribed spacer regions of ribosomal DNA (ITS1 and ITS2) is observed in many eukaryotes (e.g., vertebrates, dinoflagellates, and diatoms). The ITS1 and ITS2 regions are spliced out during the maturing process of ribosome, causing a nucleotide substitution rate higher than ribosomal DNA coding regions. Nevertheless, the ITS1 and ITS2 sequences are functionally important for their splicing, because the premature transcript composed of 18S, 28S, 5.8S rRNA, ITS1, and ITS2 is folded into a secondary structure followed by the self-splicing of ITS1 and ITS2. Based on the secondary structures of ITS1 and ITS2 sequences, compensatory base changes (CBCs: base changes occurring on both sides of a double-stranded portion) and hemi-CBCs (HCBCs: base changes occurring on one side of a double-stranded portion) are often observed among closely related species. The correlation between CBCs and HCBCs in the ITS2 sequences likely reflects sexual compatibility among individuals of a closely related species. Thus, the CBCs/HCBCs correlation is a useful marker to infer whether geographically isolated populations are reproducible.

We demonstrated that there is heterogeneity of the ITS2 sequences within an individual of *L. buetschlii* and that all individuals of *L. buetschlii* collected from the surface to deep layers in the Japan Sea do not have a significant difference in the CBCs/HCBCs of the ITS-2 sequences. Furthermore, the CBCs/HCBCs of the ITS-2 sequences do not show a significant difference between individuals of the Japan Sea and Pacific Ocean. These findings suggest that *L. buetschlii* in the Japan Sea and Pacific Ocean likely forms a reproducible single population. Thus, the geological isolation during the LGM is unlikely effective for the reproductive isolation of this radiolarian species.

Keywords: Japan Sea, *Larcopyle buetschlii*, Radiolaria, secondary deep-sea plankton