

論文内容の要旨

論文題目 **Geographic genetic structure and gene flow of three intertidal halichondrid sponges (Demospongiae, Halichondrida, Halichondriidae)**

±潮間帯に生息するイソカイメン類3種(普通海綿綱, 磯海綿目, イソカイメン科)の集団構造と遺伝子流動±

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In this thesis, to estimate geographic distribution pattern and discuss dispersal feature of intertidal sessile sponges, reproductive period, genetic structure and gene flow were investigated using the three commonest intertidal halichondrid sponges, *Hymeniacidon sinapium* (de Laubenfels, 1930), *Hymeniacidon flavia* Sim & Le, 2003, and *Halichondria okadai* (Kadoda, 1920) in Japan and Korea.

Sponge (Phylum Porifera) is primitive, evolutionary important and the group with highly diverse, though basic biological information of such as taxonomy, distribution ranges of each species, abundance, reproduction, and dispersal has not been sufficiently accumulated. Up until several years ago, sponges had been thought to be able to disperse widely across oceans during pelagic larval or gammarule phase and many sponges, even in freshwater species, had been described as cosmopolitan species or with wide distribution. However, recently, genetic analyses have demonstrated cryptic divergence and genetic separation within species, species group and also more restricted region. From general estimation of short swimming larval period and low swimming ability of larvae, short dispersal distance and more restricted distribution has been expected. These indicate that wide distribution ranges were lead from lumping or miss-identification because of paucity of morphological character and difficulty of identification.

Three studied species inhabit sympatrically in upper intertidal zone, though distribution ranges differ remarkably. *Hymeniacidon flavia* and *Hali. okadai* distribute only western North Pacific coast, while *Hym. sinapium* distributes both eastern and western North Pacific coast. It was also suggested that *Hym. sinapium* and *Hym. heliophila* from Atlantic were genetically identical. These two species also share similar morphology. Since difference in distribution range might be caused by difference in dispersal ability, reproduction periods and genetic

structures of three species were compared.

The larval morphology and reproductive period of *Hym. sinapium* and *Hym. flavia* were investigated by tissue observation and periodical sampling respectively. The reproductive period of *Hym. sinapium* and *Hym. flavia* were during warmer season of the year. *Halichondria okadai* was also known to reproduce during spring and summer. All three species had parenchymella larvae, which were entirely ciliated. The larvae of *Hym. sinapium* and *Hali. okadai* were elongated shape and that of *Hym. flavia* shaped more oval than the other two species. The larvae of two *Hymeniacion* species have "style" type spicules that arrangements were different in each species. Because the reproductive periods are almost same, influences of ocean currents over larvae that are released to a water column might be similar. Concentration and arrangement of spicule in tiny larvae might have some impact on larval dispersal.

Geographic genetic structures of three species were investigated and several significant genetic structures were observed. The three species were analyzed using sequences of internal transcribed spacers (ITS1 and 2) of nuclear rDNA and NADH dehydrogenase subunit 5 (*nad5*) gene of mitochondrial DNA. Several significant genetic structures were detected in *Hym. flavia* and *Hali. okadai*. In the both species, influences by historical geographical events after the last glacial phase and present sea current were supposed from the distribution patterns. While in *Hym. sinapium*, only a few genetic variations were detected in ITS sequence, and furthermore two Atlantic species, *Hym. heliophila* and *Hym. perlevis* had very similar sequence. It is highly likely that *Hym. sinapium* has high gene flow between distant localities and this species distribute wider range than expected. The results of the analysis using *nad5* of two *Hymeniacion* species agreed with the results from ITS analyses. Distribution patterns of *nad5* haplotypes supported high dispersal ability in *Hym. sinapium* and stable population structure in *Hym. flavia*.

For *Hym. sinapium*, 8 microsatellite markers were developed and farther detailed genetic structure was examined using seven of eight loci. Significant genetic differentiations between collection localities were detected by population genetic analyses. From a result of mantel test indicated that level of genetic differentiation had positive relation with geographic distance between localities. However, high differentiation level was also observed even between adjacent localities and the level changed slightly with distance. The result of population assignment analysis using structure program suggested that most of collection localities constitute each reproductive population. Significant genetic differentiations between collection localities suggested that natural dispersal was more likely than human mediated dispersal around Japan

and Korea. From these results, we concluded that most individuals disperse short distance like general expectation for sponges, however, there are individuals that could disperse to distant localities. Further analysis including related Atlantic and European *Hymeniacidon* species will be required to estimate the distribution origin and detailed historical demography.

In all three species, regional genetic differentiations were detected, suggesting individuals in restricted region constitute each reproductive population in the region. Influences by historical geographical events after the last glacial phase and present sea current were suggested by geographic genetic structures of these sponges. Higher dispersal ability of *Hymeniacidon sinapium* than the other species was also supported by the results. However, significant genetic differentiations were shown in *Hym. sinapium*, natural dispersal was more likely than human mediated dispersal. Further analysis including Atlantic and European *Hymeniacidon* species will be required to estimate the distribution origin and detailed historical demography.