論文の内容の要旨

Evaluation of paleotemperature proxy using coral genome biology

（サンゴゲノム生物学をつかった古水温プロキシの評価）

ベル　智子

Corals have been used as temperature proxies since the 1970's, and especially coral skeletal Sr/Ca ratios are a robust tool to reconstruct past sea surface temperature (SST). However, it is reported that corals show strong individual variability such as growth rate difference. For example, Mg/Ca ratios have been also well studied as SST proxy, but most of the studies reported lower correlations between SST and Mg/Ca ratios compared to Sr/Ca ratios. Also, Mg/Ca ratios are correlated with growth rates. This is called vital effects, and it has been addressed as a downside for using corals as temperature proxies.

In this study, I set two specific objectives. First, I focus on the question why Sr/Ca ratios excel compared to other proxies. My second objective is elucidating the vital effects at molecular level. I address these two objectives by combining geochemistry and genome biology. To achieve these two objectives, I conducted four different interactive projects. In Chapter two, we conducted culture experiments using both adult and juvenile coral of *Acropora digitifera* to verify the robustness of skeletal Sr/Ca ratios as a temperature proxy. In Chapter three, I investigated the *Acropora digitifera* genome database (Zoophytebase), and I utilize the findings from the database to understand the result from Chapter two. In Chapter four, I reported *Acropora digitifera* genes that exhibited strong vital effects from two samples with different growth rates). In Chapter five, I summarized all the results from Chapter two through four and evaluate the criteria to be effective temperature proxies.

To conduct this study, I chose *Acropora digitifera* as the study species. This species has some advantages over *Porites* sp. which has been utilized most as a temperature proxy. First, the whole genome of *Acropora digitifera* has been sequenced and published. Secondly, it has been receiving attention as a new temperature proxy and four literatures have been published. Lastly, they live in both tropics and subtropics, thus they are able to provide sea temperature records from a wide range of the ocean. Therefore, I thought this species was most ideal for my study.

In chapter two, I confirmed that Sr/Ca ratios of *Acropora digitifera* are reliable temperature proxy by conducting two different culture experiments that have not been reported previously. As adult *in situ* samples are already influenced by various environmental factors, first we conducted temperature-controlled culture experiment (20, 22, 27, and 31 °C) using primary polyps of *Acropora digitifera* to accurately assess the impact of a wide range of temperatures on the growth rate and to methodically evaluate the skeletal trace elements (Sr/Ca and Mg/Ca). Water temperatures positively affected the growth rate up to 31 °C, which exceeds the temperature threshold for this species. The growth rates also varied widely (>20%) during each of the four temperature treatments. The skeletal Sr/Ca ratios were strongly correlated with water temperature while Mg/Ca ratios showed moderate correlation ($R^2 = 0.68$, $p < 0.001$; $R^2 = 0.53$, $p < 0.001$, respectively). The variations in the skeletal Sr/Ca, and Mg/ Ca ratios at the four different temperatures were 0.36%–1.20% and 0.83%–3.76% respectively. Thus, the Sr/Ca ratios showed the least variation, despite the wide variations in the calcification rate. I suggest that the Sr/Ca ratios of *Acropora digitifera* juveniles are robust proxy of temperature, regardless of variations in the growth rate and the wide range of ambient temperatures.

Secondly, I conducted 12-month rearing experiments using 13 branches from five adult coral colonies of *Acropora digitifera* at outside aquaria (the common garden culture experiment) to compare the variations in skeletal elements observed among primary polyp samples. The sections of branches that grew during the experiment were analyzed for Sr/Ca and Mg/Ca ratios. These adult samples showed different growth rates (0.23–1.61 %/day) after the experiment. The growth rates were significantly different among colonies ($F(4, 8) = 23.898$; $p < 0.001$). I also confirmed a significant correlation between growth rates and Sr/Ca and Mg/Ca ratios. However, the correlations between skeletal elements and growth rates were - 0.69 ($p < 0.01$) and 0.24 ($p = 0.42$) for Mg and Sr, respectively. Thus Mg was largely influenced by the growth rate compared to Sr. Interestingly, the variation in the Sr/Ca ratios of *Acropora digitifera* was only 1.9%, which was one-sixth of the variation in the Mg/Ca ratios (11.9%). Thus, the influence of growth rate on Sr/Ca is much smaller than that on Mg/Ca in *Acropora digitifera* and makes Sr/Ca ratios more reliable SST proxy than Mg/Ca ratios.

In Chapter two, I could confirm that Sr/Ca ratios were correlated with water temperatures more than Mg/Ca ratios, but I was not able to explain why Mg/Ca ratios showed the lower correlation with temperature. In Chapter three, to approach this issue, I tried to establish the method to separate the vital effects from abiogenic one. In Chapter three, I utilized an open access and searchable gene database for coral Acropora digitifera, and examined the number of genes related to the elements in seawater to assess the origin of uncertainties in geochemical proxies. I found that Acropora digitifera has genes that might process at least 15 chemical elements as individual substances (Ca, Na, Zn, K, C, N, Cl, S, Fe, Mg, Mn, Cu, H, Mo, and Te) and transporters for 7 of these elements (Ca, Na, Zn, K, Cl, Cu, and H). The number of Ca-related genes was the highest (at least 428 genes, including 53 transporters), whereas Sr, one of the most widely used geochemical proxies, was not found in the gene database. Therefore, the chemical elements that exist in seawater but were not found in the gene database (e.g., Sr, Li, and U) might be processed mainly abiogenically. I suggest that elements with no relevant coral genes could be good candidates for proxies with fewer vital effects (e.g., Sr/Ca, Li/Ca, and U/Ca). For example, Li/Ca and U/Ca were proposed as useful temperature proxies, moreover, Sr-U was recently introduced as a reliable temperature proxy by combining Sr/Ca and U/Ca. Thus, the number of genes in coral genomes related to specific elements may provide at least partial criteria for determining reliable proxies.

However, in Chapter three, it was still unclear why the high correlation between Mg/Ca ratios and growth rates was observed or why the variation in Mg/Ca was the highest. For example, in Chapter two, I analyzed skeletal samples of *Acropora digitifera* exhibiting different growth rates; their Sr/Ca ratios showed the lowest variation (1.9%), whereas other Mg/Ca showed much higher variation (11.9 %). Therefore, in Chapter four, I investigated genomes that are possibly related to metabolizing skeletal elements (Sr, Mg and Ca) using two different growth rate samples showing four fold differences. Intriguingly, I did not find Sr related genes, but I identified a Mg transporter (aug_v2a.04878) that showed higher gene expressions in the fast growth sample. I suggest this gene could possibly cause growth rate difference and might explain large individual variations reported in skeletal Mg/Ca ratios, which are problematic to be a reliable water temperature proxy. Therefore this gene could be related to the phenomena called vital effects in paleoclimatology. It has been long discussed that CaATPase, which is a transporter protein, played a significant roles in coral calcification by sending Ca ions to the center of calcification site. From my results, I identified six CaATPase. Interestingly I did not find the difference in CaATPase expressions between fast and slow growth samples, thus CaATPase might not be related to the growth rate differences. Moreover, I did not find significant difference in skeletal Ca values (mol) while there was one in skeletal Mg values (mmol) from different growth rate samples. That is to say, different expressions in Ca related genes might not be affecting the skeletal Ca values while aug_v2a.04878 expression could be related to the skeletal Mg values.

It is reported that global sea surface temperature is rising about 0.53℃ per 100 years. Therefore projecting future sea temperature change is one of the critical tasks to be resolved. In this study, I was able to confirm that Sr/Ca ratios of *Acropora digitifera* of both adult and juvenile samples were robust temperature proxy. My result will be useful to reconstruct sea temperature from a wide range of the ocean. Moreover growth rate of *Acropora digitifera* is much faster than *Porites* sp. which has been widely used as temperature proxy since 1970's. Thus I can expect to collect temperature data with higher resolution.

One of the most important topics in biomineralization research is whether chemical elements in marine calcifiers are controlled biogenically or abiogenically when they are transported from seawater into the skeleton. To resolve this issue, we propose that a bioinformatics approach using genome information would be an effective method. We suggest that elements with no relevant coral genes would make good candidates for reliable proxies. Genomic information can help us find new geochemical proxies with the fewest vital effects, and also explain the robustness of geochemical proxies that are already known to be effective in reconstructing past ocean environments