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論文題目	Diversification through specialization in the algae covering molluscan shells（貝殻に付着する藻類の特殊化を介した多様化）
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論文内容の要旨

Ecological specialization is an important concept in understanding the creation and maintenance of biodiversity. Biological interactions promote ecological specialization and then lead to reproductive isolation between specialists with different ecological characteristics. In particular, symbiosis, in which species with different evolutionary histories live with each other as the partner, often has close interactions and promotes speciation through symbiont-host specialization. In addition, several symbionts can further diversify by undergoing host shift, in which they switch host use to new hosts. However, how the symbiont specializes to the host and subsequently diversify is not well understood in most symbiotic systems.

Here I focused on the green alga *Pseudocladophora conchopheria*, which grows on the shells of an intertidal gastropod *Lunella correensis*. Previous studies showed that this alga has host specificity, growing only on the shell of *L. correensis*, suggesting that a close symbiotic relationship. However, their symbiotic interactions and evolutionary history are not well understood.

I first tested the hypothesis that the alga provides the host snail with the benefit of reducing the heat stress of the host during low tide (Chapter 2). As the result, heat exposure experiments indicated that the attachment of wet *P. conchopheria* reduced heat stress of the host snail through heat of vaporization. Therefore, *P. conchopheria* would provide benefits for *L. correensis*. Also, *P. conchopheria* showed a conditional symbiosis in which the proportion of attachment depended on the environment. Thus, focusing on the fact that the relationship between the alga and the snail is well recognized by anglers or naturalists, I conducted a nationwide survey by citizen science via social media to investigate environmental factors that contribute to the establishment of symbiosis between the alga and the snail (Chapter 3). The results showed that the proportions of the algae on the shells increased with the size of the settlement substrate of the snails. *Lunella correensis* dives into the mud or sand at low tide in mud or sandy shores, suggesting that this behavior reduce heat stress. In contrast, *L. correensis* inhabiting in rocky shores may be reducing heat stress by attaching *P. conchopheria* on the shell. On the other hand, interestingly, there was no correlation between the proportion of the algae and temperature. A previous study suggested that *P. conchopheria* is neutral to the host snail. Considering that some algae are harmful to their hosts and are generalists, this result implies that the algae can adhere to shells in cooler areas where the function of heat stress reduction is less effective. Therefore, these positive and neutral interactions may have contributed to the specialization of shell utilization by *P. conchopheria*.

In the course of my survey, I found that, contrary to previous findings, *P. conchopheria* sympatricaly uses multiple intertidal gastropods other than *L. correensis* as hosts (Fig. 1). Did these algae specialize to their respective hosts and divergent genetically? To clarify this issue, MIG-seq, a genome-wide genetic analysis, was conducted (Chapter 4). The phylogenetic analyses showed that the algae were completely, or incompletely, genetically differentiated between host uses sympatrically. For example, in Kunetsu, located Amami Islands, where the algae use *Lunella granulata* and *Japeuthria cingulata* sympatrically as hosts, there was complete genetic differentiation and no current gene flow between the host use. The ancestral trait reconstructions revealed that the ancestor of the different host-use populations used the shell of *L. granulata*. Furthermore, the demographic estimation revealed that

speciation with gene flow occurred between these host uses. Thus, these results implied that speciation via host shift occurred, and that host-shift speciation would proceed gradually with gene flow.

In conclusion, positive and neutral interactions in *P. conchopheria* may have triggered specialization to shells and shifts to various hosts. *Pseudocladophora conchopheria* underwent host shifts, gradually establishing host specificity and reproductive isolation, leading to phylogenetic diversification. The symbiotic interactions with the host and mechanisms of diversification in *P. cochopheria* were clarified using by using ecological, phylogenetic and genetic methods. I emphasize that this study provides insight into understanding in the role of interactions and the speciation continuum in the ocean symbiosis.

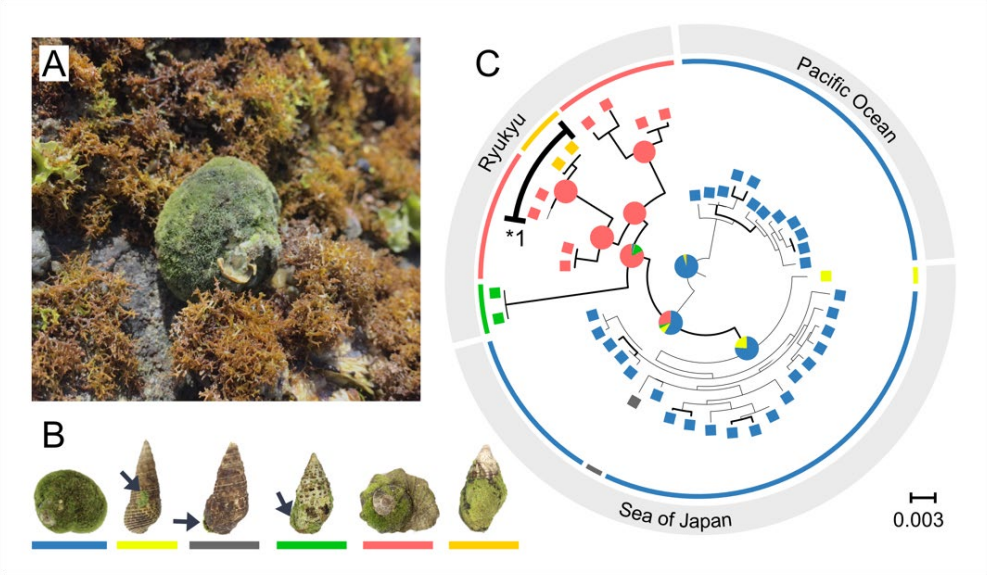

Figure 1: A indicates a photo of the green alga, *Pseudocladophora conchopheria* growing on the shell of *Lunella correensis*. B indicates *P. conchopheria* growing on the shells of various host gastropods. From left to right, are *Lunella correensis*, *Batillaria attramentaria*, *Batillaria multiformis*, *Clypeomorus bifasciata*, *Lunella granulata* and *Japeuthria cingulata.* C indicates the phylogenetic tree and the history of host utilization as revealed by ancestral trait reconstruction in *P. conchopheria*. *1 indicates Kunetsu population.

論文審査結果の要旨

香川理提出の博士論文は、潮間帯に生息する腹足類スガイとその殻に生育する緑藻カイゴロモの共生関係に注目し、カイゴロモがスガイに及ぼす利益を解明することに成功した。この 2 種の関係は古くから知られており、多くの選好研究にもかかわらず、その利益は道であった。本論文では、カイゴロモがスガイの耐温性と耐乾性を高めるのに貢献していることを、実験と野外観察により証明した。

次に上記の関係が野外でスガイとカイゴロモの双方に利益となっているかを確かめるため、ソーシャルメディアを通じた市民科学による全国調査を実施した。その結果、スガイの着生基盤の大きさに応じて、貝殻に付着する藻類の割合が増加することがわかった。スガイは干潮時に泥地や砂浜に潜ることから，この行動が熱ストレスを軽減することが示唆された. また温度分布との関係から、スガイとカイゴロモの関係には、カイゴロモからの温度耐性という正および付着自体の影響に関する中立的な相互作用が寄与している可能性があることが示された。

調査の過程で、従来の知見とは異なり、カイゴロモはスガイ以外の複数の腹足類を宿主として利用していることを明らかにした。この意外な発見に注目し、これら宿主の異なるカイゴロモが、スガイのカイゴロモからニッチシフトによって分化した系統なのかどうかを、ゲノムワイドな遺伝子解析である MIG-seq を実施した。系統解析の結果、藻類は同所的に異なる宿主利用者間で完全に、あるいは不完全に遺伝的に分化していることが示された。祖先形質の復元から、異なる宿主利用集団の祖先は L. granulata の殻を利用していたことが明らかになった。さらに、人口統計学的な推定から、これらの宿主利用集団の間で遺伝子流動を伴う種分化が起こっていることが明らかになった。これらの結果は，宿主シフトによる種分化が起こったこと，宿主シフトによる種分化は遺伝子流出とともに徐々に進行することを示唆する。

以上のように本研究は、宿主との共生相互作用と多様化のメカニズムを、生態学的手法、系統学的手法、遺伝学的手法を駆使して明らかにし、海洋共生における相互作用の役割と種分化の連続性についての理解に貢献する。

このように本論文は香川理が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって，香川理提出の論文は，博士（生命科学）の博士論文として合格と認める。