

琉球大学学術リポジトリ

ミドリイシ属サンゴにおける配偶子認識に関する研究

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論 文 要 旨

論 文 題 目 **Studies on gamete recognition in the coral *Acropora***

The simultaneous hermaphroditic coral Acroporidae are broadcast spawner. At their spawning event, many sympatric Acroporidae release their gamete bundles, thus gametes from many species are present in the water column. Although in the presence of gametes from many species, reproductive isolation is maintained. In turn, gamete recognition takes part in fertilization specificity, resulting in reproductive isolation in the Acroporidae. However, it is still obscure how gamete recognition mechanisms had been developed. In this study, here I investigate gamete recognition in the coral *Acropora* with respect to a) develop the protocol of cryopreserving sperm to perform crossing experiment between closely related species and b) how gamete recognition mechanism had been developed. Firstly, I developed cryopreservation protocols for *Acropora digitifera* sperm to reproduce without needing a genotoxic reagent. I studied cryopreservation of *Acropora digitifera* sperm with floating frames, allowing the placement of 250 μ l French straws 4 cm above the liquid nitrogen surface, resulting in a 40°C/min freezing rate. This method enabled the successful cryopreservation of sperm in 0.9 M sucrose supplemented with 20% methanol. In this protocol, I used a 1:3 (sperm:extender) dilution ratio. This finding shows beneficial effects of a sucrose solution in cryopreservation of *A. digitifera* sperm. In addition, I also developed the protocol with photocytometer to measure sperm concentration without hemocytometer. At the event of spawning, most researchers need to do crossing experiment as soon as possible. Because gamete aging affect fertilization ratio and appropriate sperm concentration for fertilization experiments is crucial, thus we need more quick protocols to measure sperm concentration prior to the experiments. With using these 2 protocols, I examined possibility of crossing within two closely related species that spawned in a different timing. If the synchronous spawning impacted the development of gamete recognition mechanism(s) to prevent hybridization, gametes from the closely related species that spawn in different timing are possible to hybridize. In *Acropora divaricata*, there were 2 morphotypes and their spawning month was September. Most Acroporidae spawns in June in Okinawa island, thus comparison between these 2 morphotypes are valuable to investigate relationship between timing spawning and development of gamete recognition; if gametes have chance to interact with gametes from heterospecifics, their gametes need to develop gamete recognition. Within 2 morphotypes (slender and robust type) of *A. divaricata*, their spawning days were not completely overlapped and crossing rates between eggs of slender type and sperm from robust type were lower than those of same morphotypes. On the other hand, crossing rates between eggs of robust type and sperm of slender type was not significantly different from same morphotypes. Robust type spawned about 2 weeks prior to slender type but small number of slender type spawned with same day and time of robust type, thus gametes from robust types do not have chance to interact with slender type. Because gametes from robust type was abundant in their spawning but those of slender type spawns in a same day of robust type need to choose small number of gametes from same morphotypes. It is possible that timing of spawning could affect the development of gamete recognition. To confirm relationship between timing of spawning and development of gamete recognition, two related species *Acropora digitifera* and *Acropora* sp1, which spawn in different months, were used to investigate the feasibility of hybridization, the similarity of polymorphic sperm proteins that are implicated in gamete recognition. The two species are structurally distinct, but nuclear sequences of partial *Pax-C* are similar based on *Fst* values and P-distance, implying that these two species speciated recently. Moreover, it is also expected that gamete recognition of these two species is similar and inter-crossing is possible to occur. To support this prediction, bi-directional hybridization between these two species using cryopreserved sperm was possible, and it is likely that these proteins probably play critical roles in species-specific fertilization.