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

Abstract

The simultaneous hermaphroditic coral Acroporidea are broadcast spawner. At their spawning event, many sympatric Acroporidea release their gamete bunldes, 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 Acroporidea. However, it is still obscure how gamete recognition mechanisms had been developed. In this study, I investigated 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 established cryopreservation protocols for *Acropora digitifera* sperm without a genotoxic reagent. For cryopreservation of *Acropora digitifera* sperm, the following protocol enabled good freezing rate (40°C/min); 1) sperm suspensions were mixed with 3 volume of 0.9 M sucrose supplemented with 20% methanol, 2) those suspensions were filled with 250 µl French straws, and then 3) froze those straws with floating frames 4 cm above the liquid nitrogen surface. In addition, I also developed the protocol with photocytometer to measure sperm concentration without hemocytometer. 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 with each other. In *Acropora divaricata*, there were 2 morphotypes and their spawning month was in September. Most Acroporidea spawns in June in Okinawa island, thus comparison between these 2 morphotypes are valuable to investigate relationship between timing of spawning and development of gamete recognition; if gametes have chance to interact with gametes from heterospecifics, their gametes need to choose conspecific gametes. 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 were 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 gemete recognition. To confirm relationship between timing of spawning and development of gamete recognition, *Acropora digitifera* and the cryptic species *Acropora* sp. 1, 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 group are 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.