

ABSTRACT

**Title: Parent-offspring relationship of the coral *Pocillopora damicornis*: the mode of planula production**

The brooding coral *Pocillopora damicornis* which is one of the most widespread corals has long been characterized as a typical planulating scleractinian (Atoda, 1947; Harrigan, 1972). The coral also demonstrated highly variable reproductive modes (reviewed in Harrison and Wallace, 1990). Stoddart (1984a; b,1988) found that asexual reproduction by means of brooded planulae was particularly important in maintain the population of *P. damicornis* in southwestern Australia and Hawaii as well as in the southern Great Barrier Reef (GBR) as reported by Ayre and Miller (2004). However, available evidence from other regions points to sexual reproduction. Population genetic surveys on eastern tropical of Great Barrier Reef showed little evidence of clonality (Benzie *et al.*,1995; Ayre *et al.*,1997) and populations are maintained by localized recruitment of sexually derived planula (Miller and Ayre, 2004). In Japan little evidences of asexual recruitment other than what could be attributed as fragmentation (Adjeroud and Tsuchiya, 1999).

In spite of the extensive studies, the role of gametes and the origin of the brooded planulae of *P. damicornis* remain equivocal. The mode of planula production in the coral is also not fully understood. Various methods were applied to investigate the origin of the brooded planulae of the coral *P. damicornis*, to address the question of broodparent-offspring relationship in *P. damicornis* in order to understand whether the offspring (or sibling planulae) are produced sexually or asexually. If planulae are produced sexually, hence planulae may not merely the clones of their broodparent colonies.

The present histological studies observed a continuous series of planula developmental stages from blastulae to planulae. The appearance of early embryos and small planulae occurred about the time that large oocytes disappeared. These observations, strongly suggest that planulae of *P. damicornis* in the Okinawan populations are derived from oocytes and are probably produced sexually.

Present studies of histocompatibility assay showed that sibling pairs always fuse with each other. All broodparent-offspring pairs except of one pair, together with an additional pairs that were brought into contact 3-4 months after planulation, also fused. Non-sibling pairs

of young colonies derived from the same broodparent combinations generally showed identical contact responses. However, some pairs from the same combination showed different responses. Moreover, when the outcomes of the three types of contact experiments (young-young, adult-young, and adult-adult pairs) were compared within the same combinations of source colonies, young colony pairs and pairs of their broodparent colonies showed different contact responses. However, the studies also revealed a high frequency of fusion between adult colonies of *P. damicornis* especially on the colonies collected from Bise and Motobu. This suggests that the degree of polymorphism at the histoincompatibility loci is markedly low in those areas. It is also likely that the genotypic diversity of *P. damicornis* has declined because of a mass coral bleaching event in 1998. Though only a few observations indicated genetic differences among siblings and between offspring and their broodparent, however, the possibility planulae was not simply as the clones of their broodparent can be suggested.

To obtain more conclusive results, different approaches using more sensitive molecular markers were conducted. However, since borrowed loci from *P. verrucosa* were used, microsatellite loci of *P. damicornis* were characterized and their variability was evaluated. The variability of the ITS1-5.8S-ITS2 region of ribosomal DNA to access its applicability to distinguish clones of *P. damicornis* was also evaluated. If either of the markers is highly polymorphic, it will be helpful to study the enigmatic mode of reproduction of the coral. In addition, PCR- DGGE (Denaturing Gradient Gel Electrophoresis) analysis of ITS1 was performed, in attempt to detect differences in ITS1 sequences between planulae and their broodparent

The present studies showed that microsatellite loci developed from *Pocillopora verrucosa* (Magalon *et al.*, 2004) were successfully amplify microsatellite of *P. damicornis* in 3 out of 4 loci previously described. However, heterozygosity and the number of alleles per locus were lower than those of *P. verrucosa*. This suggests that genetic diversity of *P. damicornis* populations may be low and that populations are maintained mainly by asexual recruits. Differ from the previous marker, application of the ITS1-5.8S-ITS2 region showed contrasting results. It showed that ITS1-5.8S-ITS2 sequences appeared to be more powerful in distinguishing closely related colonies than the microsatellite loci. In the present study, 15 colonies of *P. damicornis* showed 14 different ITS1-5.8S-ITS2 sequences. It is likely that the different ITS1-5.8S-ITS2 sequences obtained reflect true genetic variability of *P. damicornis*

colonies or at least difference in the major type of ITS sequences among colonies. When PCR-DGGE analysis was performed, in one family (Bise02#03YL) 3 planulae out of 15 planulae tested, displayed band at different position of their brood parent and the rest of their sibling planulae. This showed that PCR-DGGE analysis has possibility to distinguish clones in the coral *P. damicornis*.

During experiments investigating contact reactions in *P. damicornis*, colonies derived from primary polyps (settled planulae) and those regenerated from branch fragments of adult colonies which assumed different growth forms were observed. This difference is not due to variations in colony size since the regression lines for young-young, adult-young, and adult-adult pairs were different when the total lengths of branches were plotted against the projected area of the colonies. The capacity to initiate branches was not affected also by the contact reaction, as there was no significant difference in the number or total length of branches between fused and non-fused pairs as well. Nor to tip orientation since small colonies regenerated from adult branch fragments did not form branches regardless of the tip orientation. These observations likely implied the possibility that planulae of *P. damicornis* were produced sexually. Genetic recombination due to sexual reproduction may result in primary polyps with the capacity to originate branches. If the coral planulae are produced asexually, offspring will inherit their broodparents age, which it means that planulae will undergo aging simultaneously with their broodparent. Although remains a possibility that corals may rejuvenate if they regenerate from tissue of less than a certain size.

From this study it has been demonstrated that some planulae of *P. damicornis* from Okinawan populations were generated sexually which means that planulae were not simply the clones of their broodparents. However, the study also suggests that genetic diversity of *P. damicornis* populations are low and that populations are maintained mainly by asexual recruits. Though, this work cannot demonstrate the sexual origin of *P. damicornis* planulae completely, the present studies may support the idea that widely distributed species exhibits various reproductive mode among different regions (Harrison and Wallace, 1990; Hughes *et al.*, 1992). The possibility that *P. damicornis* consist of several species could not be rule out. Indeed, the species has been suggested as a syngameon or consist of many smaller units, which might be genetically distinct or reproductively isolated (Knowlton & Jackson 1994, Veron 1995, Ayre *et al.*, 1997).

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## 学位（博士）論文審査及び最終試験の終了報告書

学位（博士）の申請に対し、学位論文の審査及び最終試験を終了したので、下記のとおり報告します。

記

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論 文 題 目	Parent-offspring relationship of the coral <i>Pocillopora damicornis</i> : mode of planula production.	

**審査要旨（2000字以内）**

造礁サンゴの一種ハナヤサイサンゴは、研究対象としてよく用いられるものの、そのプラヌラ幼生の起源についてはまだ解明されていない。月周期に従って配偶子の成熟、プラヌラ幼生の放出が起こるが、初期胚が見つからないこと、アロザイム解析によると親群体とプラヌラ幼生は遺伝的に同じであることから、プラヌラ幼生は無性的に生じると考えられてきた。本研究では、沖縄の個体群を材料として、ハナヤサイサンゴのプラヌラ幼生の起源を明らかにすることを目的とした。

（次頁へ続く）

## 審査要旨

2章では、組織学的研究によりハナヤサイサンゴで初めて初期胚を観察し、少なくとも沖縄の個体群ではプラヌラ幼生は有性生殖により生じることを示した。ポリプ内で発生の進行するハナヤサイサンゴで初期胚を観察することは、20年以上にわたって多くの研究者が試み、なしえなかった成果である。

3章では、「組織非適合反応を示す2つの群体は互いに遺伝的に異なる」という現象を利用して、同一の群体から放出されたプラヌラ幼生間およびプラヌラ幼生と親群体間で遺伝的差異のあることを示すことを試みた。その結果、割合は少ないものの、プラヌラ幼生間およびプラヌラ幼生と親群体間で遺伝的差異のあることを示唆する反応例が少数例見られた。

4章では、さらにマイクロサテライトやリボソーム遺伝子のITS領域を用いて、プラヌラ幼生と親群体間で遺伝的差異のあることを示すことを試みた。その結果、同一親群体から放出されたプラヌラ幼生のなかに、親個体のもたない対立形質をもつ個体が2個体あることを証明できた。ハナヤサイサンゴで他家受精の起こっていることを示す初めての成果である。ただし遺伝的差異の検出される割合が低いことから、他家受精の起こる割合は少なく、自家受精や単為発生が主に起こっている可能性が示唆された。

5章では、組織非適合性試験を行う過程で発見した興味深い現象について記載した。ハナヤサイサンゴの一次ポリプ由来の幼群体は枝を形成するが、成群体から単離した枝先端部は枝を形成しなかった。この結果は、枝形成能力が発生過程により変化する可能性を示唆しており、将来の枝形成過程の遺伝子レベルの研究に道を開くものである。

本学位論文は、前期・後期課程の一貫した研究結果をまとめたものである。3, 4章は現在投稿論文を改訂中であるが、いずれ論文として発表される十分な内容をもっている。最終試験に代える口頭発表の後、2月8日午後4時30分より理530室に置いて審査会を開いて審議した結果、本学位論文は博士論文として十分な内容を備えており、合格と判断された。公開発表会における口頭発表及び質疑を見た結果、研究内容とその問題点をよく理解していることが分かり、最終試験も合格と判定された。