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Sexual and asexual production of planulae in reef corals *

D. J. Ayre [1] ** and J. M. Resing [2] ***

[1] **Australian Institute of Marine Science; PMB No. 3, Townsville M.C., Queensland 4810, Australia**
[2] **Department of Marine Biology, James Cook University; Townsville, Queensland 4811, Australia**

Abstract

Electrophoresis was used to provide genetic evidence of the mode of production of brooded planulae in each of four species of scleractinian coral collected from the central region of the Great Barrier Reef during September, October and November 1984. Comparisons were made of the multi-locus genotypes of planulae and their broodparents for two ahermatypic (non zooxanthellate) species, *Tubastraea diaphana* and *T. coccinea* and two hermatypic (zooxanthellate) species, *Acropora palifera* and *Seriatopora hystrix*. For both ahermatypic species, all planulae were found to be genetically identical to their broodparents, including 91 planulae which were heterozygous for at least one locus. These results are consistent with asexual (ameiotic) reproduction. In contrast, non-parental genotypes were detected in the majority of hermatypic broods, which is consistent with expectations for sexual reproduction with at least some outcrossing. These data confirmed that brooded planulae may be produced both sexually and asexually and countered the suggestion that electrophoretic studies of hermatypic corals may be weakened by the contaminating effect of the enzymes of their symbiotic zooxanthellae.

Introduction

Knowledge of the population biology of corals is deficient in many areas, but chief of these must be the modes of production of planula larvae and the extent of larval dispersal. It is known that planulae may be either brooded or may develop from spawned gametes (Fadlallah, 1983; Harrison *et al.*, 1984), but although direct observations of fertilization and cleavage have been made for spawning species, the mode of origin of brooded planulae has been poorly investigated. All coral larvae have been assumed to be produced sexually (Hyman, 1940), but the evidence for sexual production of brooded larvae has been derived solely from the near coincidence of brooding and gametic cycles (Stoddart and Black, 1985). A rare exception is the detection of early cleavage stages within the ovaries of *Acropora palifera* (B. Kojis, personal communication). Only one study has attempted to provide genetic confirmation of the mode of larval production. That study showed that brooded planulae of the hermatypic (zooxanthellate) *Pocillopora damicornis* were electrophoretically identical to their broodparents, a finding which is consistent with the production of brooded planulae via an unknown mechanism of asexual (ameiotic) reproduction (Stoddart, 1983).

The use of electrophoresis to study coral genetics has been limited by technical difficulties, including the presence of symbiotic zooxanthellae in hermatypic species, and the difficulty of confirming genetic interpretations of allozyme variation. Stoddart (1983) recognised that the validity of his arguments rested on the assumptions that the electrophoretic identity of the tissues of adult and juvenile *Pocillopora damicornis* did not result from contamination with the enzymes of symbiotic zooxanthellae and that correct locus-allele interpretations of zymograms could be made without the benefit of inheritance studies. These assumptions were supported by electrophoretic comparisons of normal and zooxanthellae-depleted tissue and algal pellets prepared by differential centrifugation.

In the present study, electrophoresis was used to assess the generality of Stoddart's finding of the asexual production of brooded larvae in scleractinian corals. Possible effects of algal enzymes on coral zymograms were overcome by the selection of the ahermatypic species *Tubastraea diaphana* and *T. coccinea*. Comparisons were made

* Contribution No. 306 of the Australian Institute of Marine Science

** Present address: Department of Biology, University of Wollongong, P.O. Box 1144, Wollongong, New South Wales 2500, Australia

*** Present address: Department of Zoology, Duke University, Durham, North Carolina 27706, USA

with two hermatypic species, *Seriatopora hystrix* and the apparently oviparous brooder *Acropora palifera.*

Materials and methods

Collection and maintenance of specimens

All collections were made within the central region of the Great Barrier Reef (Latitude 18° 16′–49′ S) during September, October and November of 1984 (Identifications were based on Veron and Pichon, 1976; Veron and Wallace, 1984; and Veron, 1985). Fragments of individual colonies were kept alive, at room temperature, in 1-litre containers of unaerated sea water. The sea water was changed daily and checked for the presence of planulae. Planulae were transferred to a separate container and kept alive for up to 7 d pending electrophoresis. Samples of adult tissue were frozen at −80 °C, and tissue extracts for electrophoresis were prepared from fresh or frozen material. Only actively swimming planulae were used for electrophoresis. The planulae ranged in length from > 2 mm for *Acropora palifera* to < 1 mm for *Seriatopora hystrix.*

Electrophoresis

Tissue extracts were prepared, and enzymes assayed as described by Willis and Ayre (1985). All adults and planulae were homogenised on cold plates (< 4 °C), except the small planulae of *Seriatopora hystrix,* which were homogenised at room temperature to avoid the diluting effects of condensation. All adults were assayed for the enzymes malate dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44) and superoxide dismutase (SOD, EC 1.15.1.1.) on buffer 5 of Selander *et al.* (1971), and phosphoglucose isomerase (PGI, EC 5.3.1.9) and phosphoglucomutase (PGM, EC 2.7.5.1) on buffer 9 of Selander *et al.* (1971). Individual planulae were assayed on only one buffer system and stained for a maximum of three enzymes. The enzymes MDH, PGI and PGM were all polymorphic in at least one species. With the exception of PGM for *Acropora palifera,* all isozyme patterns were similar to those described for monomeric and dimeric enzymes in other species with normal Mendelian inheritance. *A. palifera* displayed complex banding patterns for PGM, probably produced by two monomeric enzymes and satellite banding. A similar interpretation has been made for the coral *Pavona cactus* by Willis and Ayre (1985). The other three species displayed single or double-banded phenotypes for PGM consistent with a single monomeric enzyme. All specimens displayed either one or three-bands for the enzymes MDH and PGI, which were therefore inferred to be dimeric. Invariant single-bands were found for 6-PGD and SOD.

Results

Ahermatypes

The planulae of the ahermatypic *Tubastrea diaphana* and *T. coccinea* were produced asexually (ameiotically). All planulae, including the 91 offspring of 11 adults which were heterozygous for at least one locus, were electrophoretically identical to their broodparents (Table 1). This result was a highly improbable outcome for sexual reproduction. With free recombination, the genetic identity of heterozygous adults and offspring is most probable for self-fertilization; when the probability of genetic identity is 0.5. For each heterozygous adult, the probability of consistent sexual replication of its multilocus genotype can therefore be conservatively estimated as the product of the single locus probabilities

$$P_i = 0.5^{-n_i},$$

where n_i = the number of identically heterozygous planulae at the ith locus (Stoddart, 1983). The observed genotypic identity was therefore a significantly improbable outcome of sexual reproduction ($P \leqq 0.031$) for all heterozygous adults with $\geqq 5$ electrophoretically identical planulae (Table 1). The failure to detect any non-parental genotypes in the 28 planulae of four homozygous adults taken from genetically diverse populations was also consistent with asexual reproduction (Table 1).

Hermatypes

In contrast to the results for the ahermatypic species, a high proportion of non-parental genotypes was detected in

Table 1. *Tubastrea diaphana* and *T. coccinea.* Two-locus genotypes of adults of the ahermatypic corals and their brooded planulae. Estimates are given of the probability (P) that the consistent identity of heterozygous adults and their offspring could be produced by self-fertilization. na: not applicable

Species	Adults		Juveniles		P
	PGI	PGM	PGI	PGM	
Tubastrea diaphana	AB	AB	6AB	6AB	2.4×10^{-4}
	AA	AB	6AA	6AB	1.6×10^{-2}
	AB	BC	6AB	6BC	2.4×10^{-4}
	BB	BB	6BB	6BB	na
	BB	BB	6BB	6BB	na
	BB	BB	6BB	6BB	na
	–	AC	10AC	–	9.8×10^{-4}
	–	BC	10BC	–	9.8×10^{-4}
	–	BC	6BC	–	1.6×10^{-2}
Tubastrea coccinea	AA	CC	10AA	7CC	na
	AB	BC	17AB	6BC	1×10^{-7}
	AB	BC	20AB	6BC	1.5×10^{-8}
	AB	BC	10AB	6BC	1.5×10^{-5}
	AC	AB	2AC	2AB	6.3×10^{-2}
	BB	BC	6BB	6BC	1.6×10^{-2}

Table 2. *Acropora palifera* and *Seriatopora hystrix*. Two-locus genotypes of adults of the hermatypic corals and their brooded planulae

Species	Adults		Juveniles	
	PGI	MDH	PGI	MDH
Acropora palifera	CC	–	4AC, CC	–
	CC	–	5CC	–
	BC	–	3AC, 2CC	–
	BC	–	3BB, 2BC	–
	AD	–	2AA, 3AD	–
	BB	AB	5BB, BD	2AA, 2AB, 2BB
	BB	BB	4BB, BD	6BB
	BB	–	6BD	–
	BD	AB	3BB, BD, 2DD	2AA, 4AB
	BD	–	3BB, 2BD, DD	–
	DD	AB	6DD	3AA, 3AB
Seriatopora hystrix	PGM	PGI	PGM	PGI
	AB	AA	2AA, 4AB, 3BB	12AA, 2AB

the broods of adult *Acropora palifera* and *Seriatopora hystrix*. For *A. palifera*, 10 of 11 broods contained non-parental genotypes, and 5 broods contained novel alleles (not found in the broodparent; Table 2). These data are consistent with the sexual production of brooded planulae involving at least some cross-fertilization. The numbers of planulae assayed from individual adults were too small to permit tests of the probability that the genotypic composition of broods could have resulted from single matings. However, no broods displayed more than two allelic forms of the enzyme PGI, for which four alleles were present within the population. The single brood of *Seriatopora hystrix* included planulae with non-parental genotypes for both PGI and PGM (Table 2). The known parent was an AA homozygote for PGI, but two of its 14 brooded planulae were AB heterozygotes (Table 2). This represents a significant departure from the 1:1 ratio of homozygotes to heterozygotes ($\chi^2_{(1)} = 5.8$; $P < 0.02$) which would be the predicted outcome for fertilization by a single AB heterozygote, and implies that the brood was produced either by fertilization of the broodparent by two or more colonies or by a mix of cross- and self-fertilization.

Discussion

Electrophoretic comparisons of adult *Acropora palifera* and *Seriatopora hystrix* and their brooded planulae provide the first genetic evidence in support of the long-held belief that coral planulae can be produced sexually, by cross-fertilization. However, this study also demonstrated the asexual production of brooded planulae in the ahermatypic *Tubastrea diaphana* and *T. coccinea*. The latter finding is supported by similar results for the hermatypic *Pocillopora damicornis* (Stoddart, 1983).

An important feature of the present study is the fact that it greatly strengthens existing arguments for the use of electrophoresis in the study of the population genetics of corals (Stoddart, 1983; Willis and Ayre, 1985). This study provides the first evidence (based on half-parent genetics) for the segregation of alleles, at enzyme encoding loci, in scleractinian corals. This result cannot easily by explained by the "inheritance" of symbiotic zooxanthellae or their enzymes or the enzymes of the broodparent, and is consistent with the histological evidence of internal fertilization in *Acropora palifera*. Furthermore, the levels of enzyme activity and enzyme polymorphisms detected for the ahermatypic *Tubastrea diaphana* and *T. coccinea* were similar to those reported for hermatypic species. These findings, together with the recent report of diploidy in each of four families of scleratinian corals, including the Acroporidae (Heyward and Babcock, 1986), suggest that electrophoretic data can be tested against the predictions of existing genetic models for diploid species.

Preliminary electrophoretic studies have revealed patterns of genotypic diversity consistent with the predicted effects of predominantly sexual recruitment into populations of *Seriatopora hystrix* (Resing and Ayre, 1985) and *Acropora palifera*, and predominantly localised asexual recruitment into populations of *Tubastraea coccinea*. These are the outcomes which would be predicted if brooded planulae made up the majority of local recruits. Nevertheless, further studies may reveal alternative modes of larval production in some or all of these species. In particular, the two ahermatypic species might be expected to reproduce sexually. During this study, a high proportion of colonies contained ova, and *T. coccinea* was found to be a simultaneous hermaphrodite (Ayre and Resing, in preparation), and studies of other coelenterates suggest that gonads are unlikely to be involved in the asexual production of brooded larvae. This is true for the coral *Pocillopora damicornis* (Stoddart and Black, 1985) and the sea anemone *Actinia tenebrosa* (Ayre, 1984 a). Further, Cutress (1979) has described the development of brooded larvae from ingested tentacles in sexually mature adults of the sea anemone *Bunodeopsis medusoides*. In fact, the patterns of genotypic diversity revealed by electrophoretic studies of populations of both *P. damicornis* (Stoddart, 1983, 1984) and *A. tenebrosa* (Black and Johnson, 1979; Ayre, 1983, 1984 b) have been used to infer life-histories which involve the localised recruitment of asexually produced brooded juveniles and the more widespread dispersal of sexually produced, genetically diverse, colonists. This reproductive strategy satisfies many theoretical predictions for the roles of sexual and asexual reproduction (Williams, 1975; Bell, 1982) and may be applicable to *T. diaphana* and *T. coccinea*. There is currently no reason to suspect any alternative mode of larval production in *A. palifera* or *S. hystrix*, although both species may undergo some asexual reproduction by fragmentation (Highsmith, 1982). Further electrophoretic studies could be used to test for evidence of both sexual and asexual recruitment and to determine the scales of larval dispersal for each of these species.

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