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Life Cycle of the Multiarmed Sea Star *Coscinasterias acutispina* (Stimpson, 1862) in Laboratory Culture: Sexual and Asexual Reproductive Pathways

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The multiarmed sea star *Coscinasterias acutispina* generally has 7–10 arms and 2–5 madreporites. It is known to be able to reproduce by asexual fission, and we have previously observed that this species also has the ability to reproduce sexually; however, there has been no report until now of spawning in this species. We succeeded in establishing a long-term culture of juveniles produced by artificial fertilization. Twelve months after the completion of metamorphosis, three individuals had six arms of the same length and a madreporite. At this time, fission occurred in two of these individuals, while the remaining individual underwent fission four months later. Each sea star divided into two halves, provided with three arms each. Thereafter, four or five new arms and two or four madreporites were formed anew in each of the six daughter sea-stars, so that by 30 days after the first fission the number of arms and madreporites in each was similar to that in adults. A second fission occurred in four of these six individuals, four or five months after the first fission, and in three of them the plane of division was the same as that of the first fission. The original three individuals eventually proliferated to 12 by undergoing fission. All individuals had fully developed gonads by 1–3 months after the second fission. Some of them eventually spawned under laboratory culture, and the resulting larvae metamorphosed into juveniles. Our observations demonstrate that individuals of *C. acutispina* possess the potential for both sexual and asexual reproduction.

Key words: asteroid, spawning, metamorphosis, development, regeneration, reproduction

INTRODUCTION

It is well known that a number of species of benthic marine invertebrates can reproduce both sexually and asexually (Hughes, 1987). Asexual reproduction of these species commonly involves a single individual dividing into two parts; thereafter, each part regenerates the lost part and becomes a complete individual. Classes of echinoderms that include asexually reproducing species are the asteroids, ophiuroids, and holothuroids (Mladenov, 1996).

Approximately 1,800 extant species of sea stars have been described (Hendler et al., 1995). Sea stars generally reproduce sexually (McEdward and Miner, 2001), but 24 species are known to reproduce asexually by fission (Emson and Wilkie, 1980; Mladenov, 1996). *Coscinasterias acutispina* is one of these (Yamazi, 1950). Its adults generally have 7–10 arms and 2–5 madreporites (Fig. 1A, B).

Adult individuals of both *C. acutispina* and *C. muricata*, another fissiparous sea star, have fully developed gonads, and the entire course of development from egg to juvenile has been observed following artificial fertilization in the laboratory (Barker, 1978; Shibata et al., 2010); it has therefore been suggested that these two species of *Coscinasterias* must have the ability to reproduce sexually in nature as well (Shibata et al., 2010). Both species show indirect development via bipinnaria and brachiolaria larvae. Newly metamorphosed juveniles of *C. acutispina* have six arms and one hydropore (Shibata et al., 2010). The population genetic structure of *C. acutispina* has been investigated around Japan in order to clarify the contributions of both sexual and asexual reproduction to population maintenance. Haramoto et al. (2006) used random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers to detect clones arising by fission and to assess gene flow between sites. A simulation approach using these data revealed the presence of clonal individuals produced by asexual reproduction at almost all sites. The phylogenetic relationships among RAPD genotypes indicated the existence of gene flow between the sites, and Haramoto et al. (2006) suggested that this gene flow might be due to the dispersal of planktonic larvae produced by sexual reproduction; however, spawning of *C. acutispina* has never been reported in the laboratory or in the field. In the present study, we cultured juveniles obtained by artificial fertilization and observed fission, regeneration, maturation, and spawning in

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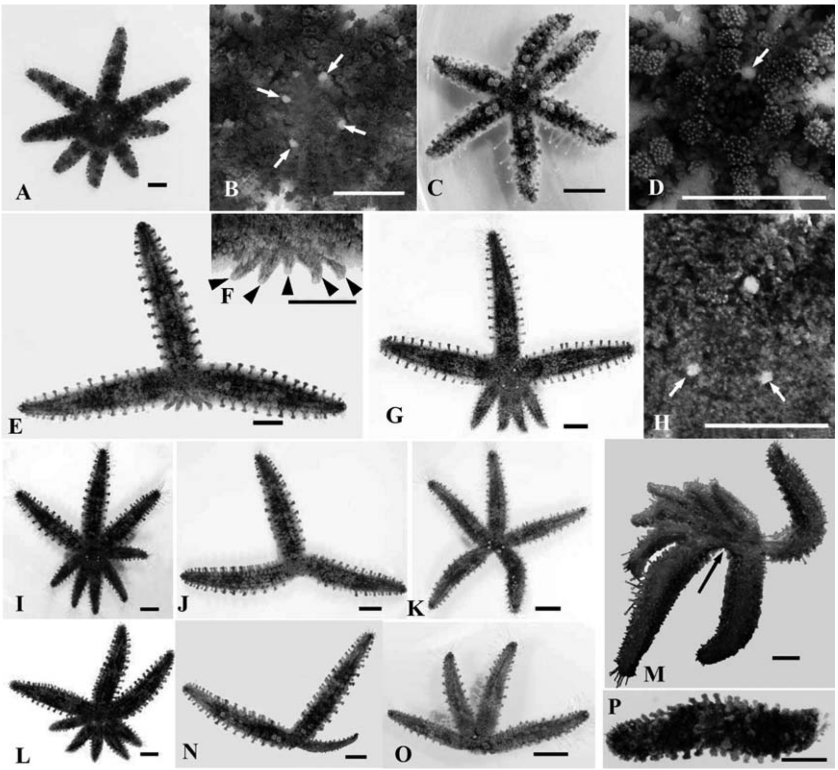

Fig. 1. A wild-caught adult and two fission-generations of cultured specimens of *Coscinasterias acutispina*. **(A)** Adult, aboral view. **(B)** Magnified view of disk in (A), with arrows indicating madreporites. **(C)** Individual 12 months after the completion of metamorphosis, aboral view. **(D)** Magnified view of disk in (C) with arrow indicating madreporite. **(E)** Individual without a madreporite one month after the first fission, aboral view. **(F)** Magnified view of five new arms (arrowheads) in (E). **(G)** Individual with madreporites two months after the first fission, aboral view. **(H)** Magnified view of disk in (G), arrows; new madreporites. **(I)** Individual four months after the first fission, aboral view (Ab in Fig. 2). **(J)** Individual with three long arms two days after the second fission in (I), aboral view (Aba in Fig. 2). **(K)** Individual with five short arms two days after the second fission, aboral view (Abb in Fig. 2). **(L)** Individual four months after the first fission, aboral view (Ba in Fig. 2). **(M)** Specimen in process of reproducing asexually by the second fission, oral view, with long arrow indicating the plane of fission. **(N)** Individual with two long arms and one short arm two days after the second fission, aboral view (Baa in Fig. 2). **(O)** Individual with four short arms two days after the second fission, aboral view (Baba in Fig. 2). **(P)** Individual with one long arm two days after the second fission, aboral view (Babb in Fig. 2). Scale bars: 10 mm.

the fissiparous multiarmed sea star *C. acutispina*.

MATERIALS AND METHODS

Adults of *C. acutispina* were collected on the coast of Kominato, Chiba, Japan, in July, 2005. The ovaries were dissected and treated with 1-methyladenine (10^{-6} M) for 30 minutes to obtain mature ova (Kanatani, 1969). A dilute sperm solution was prepared from pieces of mature, excised testis. Using these materials, artificial insemination was performed. After embryos had hatched from the fertilization membrane, the larvae were cultured until completion of metamorphosis in accordance with the method of Shibata et al. (2010). The standard temperature for development was 19°C, which is the seawater temperature at the sampling site during the breeding season. Newly metamorphosed juveniles were cultured in a tank, 30 cm tall and 25 cm in diameter, containing stones with attached individuals of the annelid Spirorbidae *Dexiospira brasiliensis* (Grube, 1871) as food. The seawater in the tank (14 liters) was stirred (30 rpm) continuously and changed once a week. Thereafter, juveniles with a radial arm length of more than 4 mm were fed living individuals of the gastropod Littorinidae *Littorina brevicula* (Philippi, 1844). The distance from the mouth center to the arm tip (R) of all arms was measured with an ocular and vernier micrometers, and the longest arm lengths were used for an index of body size. Larvae and juveniles were observed with binocular compound (BX51, Olympus, Japan) and stereoscopic dissecting (SZX9, Olympus, Japan) microscopes.

At 18 months after the completion of metamorphosis, a small incision was made on the dorso-lateral side at the base of a long arm of each surviving individual using scissors, and a piece of the gonad was excised. For microscopic observation, gonads of cultured specimens were fixed in Bouin's solution, dehydrated in an ethanol series, and embedded in paraffin wax. Sections 8 µm in thickness were then serially cut and stained with Delafield's hematoxylin and eosin.

RESULTS

Newly metamorphosed juveniles with six arms were 348.8 ± 10.8 µm in R (mean ± SE, n = 10, min. = 324.5, max. = 360.5), as has already been reported (Shibata et al., 2010). By 12 months after the completion of metamorphosis, three young individuals (A–C in Fig. 2) had six arms of the same length (Fig. 1C) and a madreporite in an interradial area between two arms (Fig. 1D). R in these specimens was 39.5 mm, 39.7 mm, and 40.5 mm, respectively. At this time, two individuals (A and B in Fig. 2) each divided into two equal-sized halves, which are labelled Aa, Ab, Ba, and Bb in Fig. 2, each half possessing three arms. The remaining individual (C in Fig. 2) reproduced asexually and equally by fission four months later (i.e. 16 months after the completion of metamorphosis), and each of its two daughter sea stars (Ca and Cb in Fig. 2) had three arms. Three of the six individuals produced to this point by fission, namely Aa, Bb, and Ca (Fig. 1G), had a madreporite, while the three, namely Ab, Ba, and Cb (Fig. 1E), lacked one, as shown in Fig. 2. Thereafter, each of these six individuals regenerated two arms first, and then two additional new arms were formed between the pair of primary regenerated arms. Furthermore, five of these individuals, excluding Ca, formed one more new arm between the pair of secondary regenerated arms (Fig. 1F). Either two or four new madreporites were formed in each of six individuals, as shown in Fig. 2. By one month post-fission, these six individuals all had seven (Fig. 1G) or eight arms (Fig. 1E, F) and 2–4 madreporites (Fig. 1H). Thereafter, they were fed a gastropod, *L. brevicula*, and the regenerated arms of these specimens grew rapidly. By two months post-fission (Fig. 1G), R of the original arms was 50.9 ± 0.8 mm (mean ± SE, n = 6, min. = 50.5, max. = 51.5), and that of the arms regenerated after fission was 20.0 ± 0.7 mm (mean ± SE, n = 6, min. = 19.5, max. = 20.5). By four months post-fission (Fig. 1I, L), R of the regenerated arms

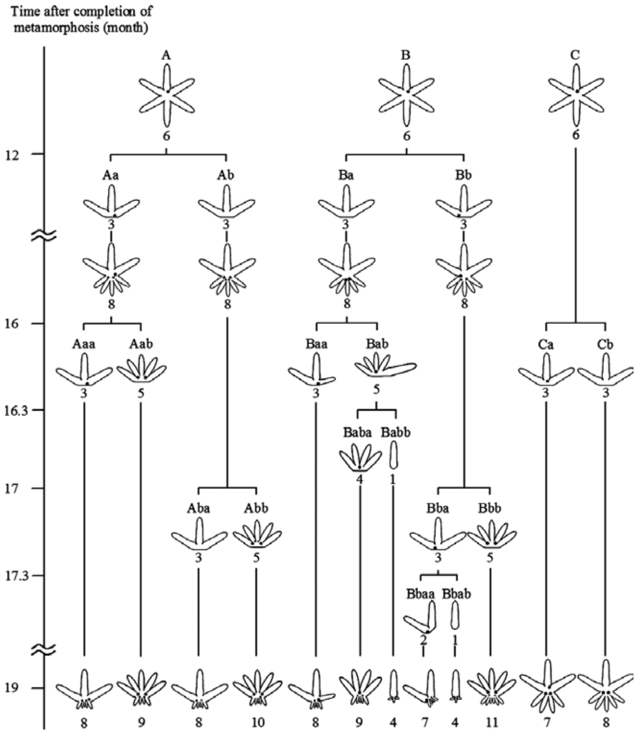

Fig. 2. Timing of the occurrence of successive fissions in individuals of *Coscinasterias acutispina* obtained by artificial fertilization. The letters above the drawings denote each specimen's code, as employed in the text. The numbers beneath the drawings represent the number of arms of each specimen. Dots in disks indicate madreporites.

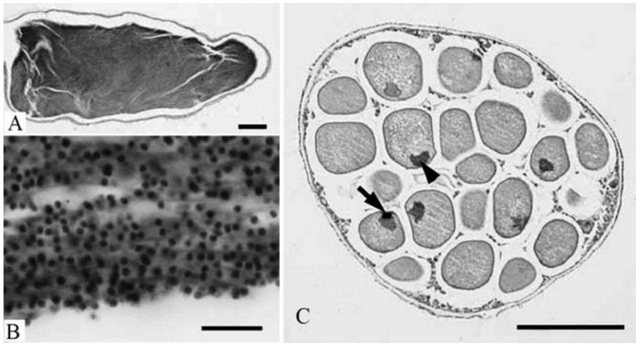

Fig. 3. Histological sections of the gonads of a male and the female of *Coscinasterias acutispina* 18 months after the completion of metamorphosis. **(A)** Testis from a long arm of individual Ca in Fig. 2. **(B)** Magnified view of testis in (A) black spheres are heads of sperm. **(C)** Ovary from a long arm of individual Baa in Fig. 2; arrowhead, germinal vesicle; arrow, nucleolus. Scale bars: A, C = 200 μm; B = 10 μm.

was 28.3 ± 0.5 mm (mean ± SE, n = 6, min. = 27.5, max. = 29.0), half that of the original arms.

Four of these six individuals (Aa, Ab, Ba, and Bb) reproduced asexually by a second fission four or five months after the first fission. In three of them, excluding Ba, the division plane of the second fission was the same as that of the first. This is shown by the fact that three of the newly produced individuals (Aaa, Aba, and Bba in Fig. 2) had only the long original arms (Fig. 1J), and the other three (Aab, Abb, and Bbb in Fig. 2) had only short arms regenerated after the first fission (Fig. 1K). The products of the fission of Ba (Fig. 1M), in contrast, included one daughter individual (Baa in Fig. 2) with two long original arms and one short, regenerated arm (Fig. 1N) whereas the other daughter sea star (Bab in Fig. 2) had one long original arm and four short, regenerated ones. Eight days after the second fission of Ba, Bab divided again into two individuals, one with just the one long arm (Babb in Fig. 2) and the other with the four short arms (Baba in Fig. 2) (Fig. 1O, P). Eight days after the second fission of Bb, one individual among the three with long original arms (Bba in Fig. 2) divided further into two individuals, one with one arm (Bbab in Fig. 2) and the other with two arms (Bbaa in Fig. 2).

Four individuals underwent both second and third fissions, giving rise to a total of 10 individuals designated Aaa, Aab, Aba, Abb, Baa, Baba, Babb, Bbaa, Bbab, and Bbb in Fig. 2. In seven of these individuals, excluding Babb, Bbab, and Bbb, two regenerating arms appeared first, and two more regenerating arms arose secondarily between the primary regenerated ones, as was the case following the first fission. In addition, one new arm was formed between a pair of secondary regenerated ones in these seven individuals. In Bbb, starting with five short regenerated arms, two primary and two secondary regenerated arms were formed similar to those in the mentioned seven individuals, but thereafter two, not one, additional new arms formed. In Babb and Bbab, each starting with only one long original arm, two new arms appeared first, and then one new arm was formed between the initial arms. Thus, the original three individuals we studied gave rise to a total of 12 through a series of up to three fissions, as shown in Fig. 2. All individuals eventually regenerated 3–6 new arms and one or four madreporites, thus acquiring a total of 4–11 arms and 2–5 madreporites.

Seventeen to 19 months after the completion of metamorphosis, some of these 12 individuals kept continuously in the large culture vessel shed gametes. At this time the testes of individuals Ca and Cb (Fig. 2) were filled with sperm (Fig. 3A, B), and the ovaries of the remaining individuals contained fully-grown eggs (Fig. 3C). By one month after this spontaneous spawning, the resulting embryos had developed into brachiolariae via the bipinnaria stage. Thereafter, these brachiolariae metamorphosed into juveniles. The newly metamorphosed juveniles had six arms, each with two pairs of tube-feet and one hydropore.

DISCUSSION

The mechanism responsible for induction of fission in asteroids has remained obscure (see Chia and Walker, 1991). Something is known, however, about the regulation of regeneration. In *Asterias vulgaris*, single arms without a madreporite have been reported to be incapable of regenerating the rest of the animal, whereas regeneration of the missing parts can occur in exceptional cases if a piece of the disk containing only the madreporite and the stone canal remains attached to an arm (King, 1900). However, the tropical sea-star *Linckia multifora* reproduces by autotomy of arms, the cast-off single arms, with no madreporite, being called “comets” owing to their form after the regeneration of other arms has begun (Edmondson, 1935; Hirota, 1985). Rideout (1978) reported that individuals in the comet phase represented the highest percentage among population samples collected throughout the year. In the first fission of our cultured *C. acutispina*, each of three individuals with six arms and one madreporite divided into two new individuals. Three of these six resulting half-stars had a madreporite; the remaining three did not, yet they regenerated four or five new arms and two or four madreporites, a result little different from that of the former three and also similar to the morphology of wild adults (Yamazi, 1950). This shows that individuals of *C. acutispina* with no madreporite and one arm can survive and regenerate in the same manner as autotomized arms of *L. multifora*.

In the present study, three individuals of *C. acutispina* obtained by artificial fertilization eventually propagated themselves into 12 as a result of fission. In the second fission, almost all daughter sea stars received at least one madreporite. Yamazi (1950) also noted this, and he also observed that this species has a distinct fission plane. We confirmed this latter observation in three of four individuals with madreporites undergoing the second fission, in which the fission plane was the same as in the first fission; however, our remaining individual divided along a different plane that its original one. Fujita (1999) found that the *C. acutispina* population in the field consisted predominantly of 8-armed individuals with four long arms and four short, regenerated ones. As noted above, the metamorphosed juveniles of this species have six equal-sized arms; therefore, individuals found in the field with more than seven arms have evidently undergone at least one fission. Conversely, there can be little doubt that any individual collected in the field with one madreporite and six arms had been produced sexually.

The sex ratio in any given population of *C. acutispina* is unequal (Seto et al., 2000), and individuals with regenerating arms are always observed. Mature males or females often account for a very small proportion of the population, and gonads are seldom well developed in individuals with regenerating arms. However, fertilized eggs have been obtained by artificial fertilization, and these metamorphosed into juveniles (Shibata et al., 2010). In the present study, individuals obtained by artificial fertilization spawned naturally in the aquarium, and the fertilized eggs developed into juveniles through metamorphosis. This is the first time that spawning following fission in an individual obtained by artificial fertilization has been demonstrated for fissiparous asteroids. Therefore we suggest that some adults develop gonads, shed gametes, and produce new individuals in the field. Fig. 4 shows the assumed reproductive cycle of this species. Adults shed gametes, and the embryos obtained by spawning develop into brachiolariae via the bipinnaria stage. These brachiolariae then metamorphose into juveniles with six arms and a hydropore. These juveniles grow and reproduce asexually via initial fission. Individuals resulting from the first fission regenerate arms and madreporites. Thereafter, individuals undergo repeated fission and become similar to adults, with eight arms, including four short, regenerated ones, and four madreporites. In addition to sexual reproduction, they can reproduce asexually by fission to increase the number of individuals (by up to three times in our study). Thus, this species reproduce sexually and produce new individuals in the field. Our present findings suggested that *C. acutispina* populations are maintained by both sexual and asexual reproduction.

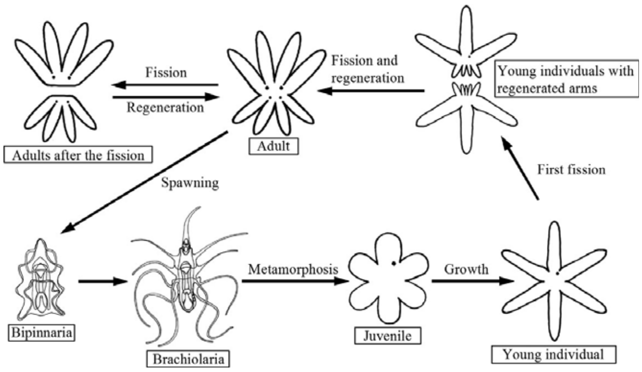

Fig. 4. Sexual and asexual pathways in the reproductive cycle of *Coscinasterias acutispina* deduced from the present and previous studies. Dots in disks indicate madreporites. Planktotrophic brachiolariae obtained by sexual reproduction settle to the substratum and metamorphose into juveniles, while individuals including the young undergo regeneration following division, thus increasing their number.

For the culture of juvenile echinoderms, feeding is an essential point to consider. Until now, no information has been available about which organisms can serve as food for asteroid juveniles in culture, except for *Acanthaster planci* (Lucas and Jones, 1976) and *Stichaster australis* (Barker, 1979). In both of these species, juveniles smaller than 8 mm in diameter feed on coralline algae, while adults of each feed on coral polyps and gastropods, respectively. Thus, the diet of these asteroids differs markedly between juveniles and adults. Fujita and Seto (1998) reported that adults of *C. acutispina* are one of the major predators of gastropods such as abalone and horned turban. In the present study, small juveniles of *C. acutispina* fed on the annelid *Dexiospira brasiliensis* two days after the completion of metamorphosis. Larger juveniles of R greater than 4 mm fed on gastropods, and could thereby be cultured in the laboratory for up to 20 months after the completion of metamorphosis, growing to a size exceeding R = 40 mm. We have thus shown that both juveniles and adults of this species are carnivorous, with juveniles feeding on tubicolous annelids, and adults generally on gastropods. These results differ from those reported for *A. planci* and *S. australis*, the juveniles of which are herbivorous, and only the adults carnivorous. In asteroids the distribution and habitat of juveniles and adults differ as already reported in *A. planci* (Zann et al., 1987; Sumida et al., 2001). One reason for this may be their different food requirements, as pointed out by Sumida et al. (2001). Juveniles of *C. acutispina* have never been observed in the field, presumably because they are cryptic. The trophic role during the juvenile phase revealed by the present study may make it easier to locate them, leading to clarification of the ecology of *C. acutispina*, including its reproduction and population dynamics.

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