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Autotomy and Regeneration in Japanese Sea-stars and Ophiurans

I. Observation on a Sea-star, *Coscinasterias acutispina* STIMPSON and Four Species of Ophiurans[1]

With 4 Text-figures

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(*Communicated by* T. KOMAI)

In *Echinodermata*, pentamerous symmetry of the body is of general occurrence. Certain species of sea-stars and ophiurans, however, have more than 5 arms. This is often correlated with autotomous division and regeneration of the animal. I have made some observations on this process occurring in a sea-star *Coscinasterias acutispina* and four species of ophiurans, all of which are found very commonly in the littoral of Japan.

Before going further, I offer here my hearty thanks to Prof. T. KOMAI, Prof. D. MIYADI and Dr. H. UTINOMI, for their kind encouragement, and also to Dr. S. MURAKAMI for the gift of the specimens of the ophiurans described in this paper.

OBSERVATIONS ON *Coscinasterias acutispina* STIMPSON

(1) Self-division across disc

LUDWIG (1897) and CROZIER (1915) carried out a statistical study on the number of rays in *Asterias* (*Coscinasterias*) *tenuispina* which lives in the Mediterranean Sea and in the Atlantic Ocean near Bermuda. They found that the number of rays was different in different localities; in some localities 6-rayed specimens were prevalent, while in others

1) Contributions from the Seto Marine Biological Laboratory, No. 135.

there were many 7-rayed ones. EDMONDSON (1935) has pointed out that the 7-rayed state seems to be typical of *C. acutispina* found in Hawaiian localities. I gathered many specimens of *Coscinasterias acutispina* on the beaches of Kada and Seto in Wakayama Prefecture, where this sea-star is commonly found under stones in the intertidal zone from spring to autumn. The specimens were collected at the same places in three years, from 1945 to 1949, and altogether about 1000 specimens were obtained. These individuals were divided into 8 classes according to the number of rays as shown in Table 1.

Table 1.

The specimens with different number of rays.

Form	Number of individuals	%
4-rayed	77	8
5- ,,	6	0
6- ,,	12	1
7- ,,	34	4
8- ,,	813	79
9- ,,	72	7
10- ,,	9	1
11- ,,	2	0
Total	1025	100

It is thus evident that the 8-rayad state is predominant in these localities, and the 7-, 9- and 10-rayed ones are to be regarded as individual variations of the 8-rayed state; the individuals with 4 rays are probably those which were divided recently, and have not yet regenerated the lost half. In the specimens having from 7 to 10 rays, two classes of rays different in the degree of growth can be recognized. This view has been substantiated by a careful observation on the superficial structure and also by dissection of the specimens. The distinction of two classes of rays may be recognized only in young stages having arm-length from 20 to 50 mm. In more developed individuals, owing to the rapid growth of regenerated rays, it is generally difficult to make a distinction between the old and new arms. It is also evident that the potency of self-division and regeneration is greater in young stages than in old ones. In young specimens from 1 to 10 mm in arm length, the axis of separation and the number of regenerated rays are different from those in larger specimens. Such younger specimens will be described in another report. Most of the slightly older specimens (20–50 mm in length of arms), as mentioned above, have the original 4 and the regenerated 4 or 5 rays. The specimens with 5 regenerated rays acquire a supernumerary ray later in the middle of the 4 previously-formed rays.

CROZIER (1915) reports in his observation on *C. tenuispina* that an individual with 7 rays divides into two parts, one with 3 rays and the other with 4 rays, and in each, 4 rays (rarely 5, 3, or 2 rays) are regenerated. This is the reason why 7- and 8- rayed individuals are nearly equally common. More precisely, however, the 7-rayed state is more prevalent; this is because many individuals do not practice their regeneration all throughout their lives. In the case of *C. acutispina*, most of the individuals have 8 rays, and every individual has a "breaking joint" across the dorsal disc, so that it is natural that the 8-rayed state is common in this species. As mentioned above, self-division is carried out more actively in young stages. By the time the regenerated rays have grown to the same length as the original ones, the sea-star usually acquires fully developed gonads. This fact indicates that the self-division is a kind of asexual reproduction. The self-division of this sea-star seems to occur in all seasons, but most commonly in summer. Undoubtedly the same individual undergoes division several times in its life.

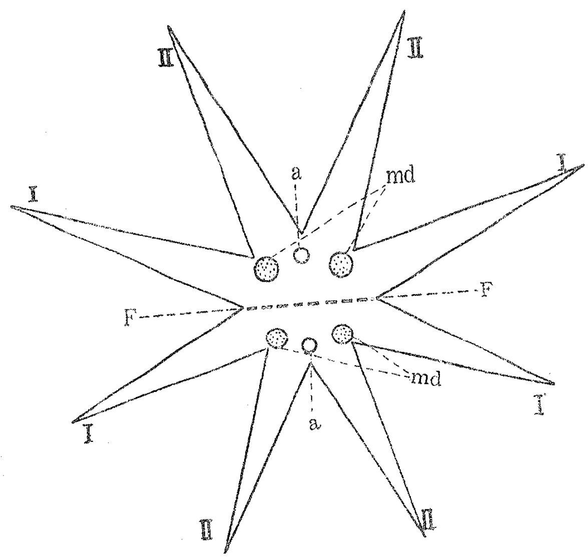

Fig. 1. Diagrammatic figure of *Coscinasterias acutispina*, viewed from abactinal side, showing the position of 4 madreporites (*md*), 2 anal openings (*a*) and the line of fission (*F*–*F*). I and II signify the primary and secondary rays respectively.

According to CROZIER (1920), *C. tenuispina* possesses a breaking joint on the arm between the 4th and 5th ambulacral plates, although no special structure serving for this purpose is found. When the sea-star is pulled loose from the substratum and placed out of the water, or when the water is very turbid, it often divides spontaneously through this breaking joint. Many individuals with injured discs may be found after heavy storms, but I have never found any isolated rays without discs. From this fact I surmise that the breakage takes place as the animal's physiological reaction to sudden change of environmental conditions, and it is not caused by mechanical injury.

The isolated arm contains a hepatic caecum, and, if of old and mature individuals, gonads as well. STUDER (1876) states that the breakage causes deterioration of the sexual cells. However, this is not true for this species. In the natural condition, isolated rays deprived of a part of the disc generally die within a few days without showing any sign of regeneration. For regeneration, the piece must retain a part of the disc, as universally recognized by previous investigators. The spontaneous division across the disc is known in species of *Sclerasterias*, *Stephanasterias*, *Coscinasterias*, *Asterias*, etc., through works by KING (1898, 1900), CROZIER (1915, 1920), FISHER (1925), BENNETT (1927) and EDMONDSON (1935). The presence of relatively many madreporites as a factor favouring spontaneous division, as suggested by many investigators, is supported by my observations on the Japanese specimens.

As shown in Figs. 1 and 2 A, a typical specimen of *C. acutispina* posesses two pairs of madreporites and a pair of anal openings arranged symmetrically on each side of the line of fission (F-F). The madreporites are placed between the I and II rays, and each anus is placed between the paired II. The position of the madreporites evidently determines the plane of fission. If fission did not occur along this line (F-F), it should pass along the line of the anus or the line of the madreporites, and it should divide the anus or the madreporites. As a matter of fact, no individual suggesting such fission has been observed. The line of fission is a shallow furrow, and never cuts across a plate. The body wall in this furrow is formed only by epidermis and contains fewer reticulated tissues than other parts. Of muscles, those running along the line of fission are prominent, and those passing across the furrow are very few.

Of the digestive organs, the anus, rectum and the rectal sac are placed very close to the margin of the disc, instead of being nearly in the center as in most other sea-stars. The pyloric portion of the stomach is very thin, and has none of the wrinkles which appear very prominently in the other parts. The cardiac portion is also divided in a complicated manner into two by the line of fission. On the whole, the internal structure also is well adjusted for spontaneous separation.

The regeneration of new arms takes place symmetrically in relation to the line of previous fission. Two rudiments of new rays (I) appear first, then those of the other two arms (II) appear between the former (Figs. 2 D and 3). Normally two ani are present, exceptionally three. Likewise, the madreporites are normally four in number, exceptionally three. It is well-known, through the works of FIELD (1892), GEMMILE (1916), NEWMAN (1921 a, b) and others, that two hydro-

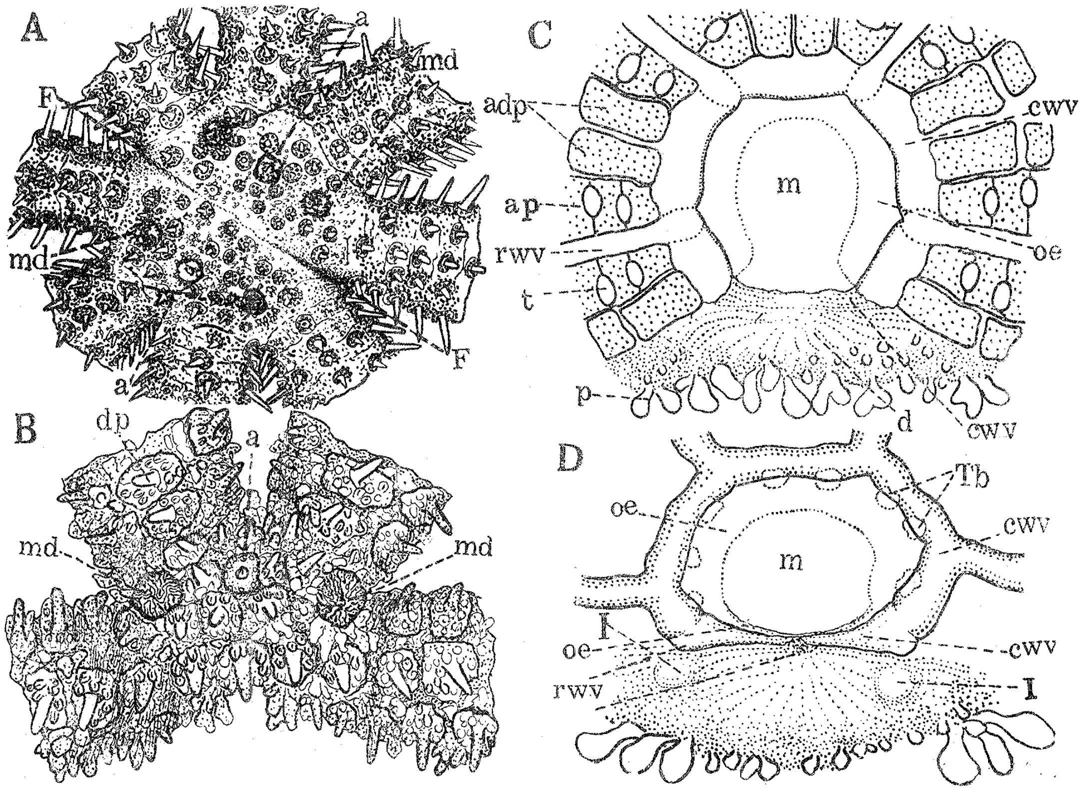

Fig. 2. (A) Normal individual before division, viewed from abactinal side.
(B) Dorsal disc soon after division, showing dorsal disc and adjoining arms curved towards injured side (basal side in the figures).
(C) Actinal side after division, showing narrowed injured surface and newly formed tissue.
(D) Actinal side, at the time when the rudiments of first rays (I) appear.
a anus; *adp* adambulacral plate; *ap* ambulacral plate; *cd* common duct of pyloric caeca; *cps* cardiac portion of stomach; *cwv* circumoral water-vessel; *m* mouth; *oe* oesophagus; *os* ossicle; *p* papula; *pp* pyloric portion of stomach; *rs* rectal sac; *rwv* radial water-vessel; *sc* stone-cannal; *Tb* Tiedemann's body. Other legends as in Fig. 1.

coels are formed symmetrically in an early developmental stage of asteroids, and that the one on the right side degenerates and that on the left side grows to the water-vascular system of the adult. This fact suggests the possibility of the formation of symmetrical hydrocoels in some exceptional cases.

Although the paired digestive system as well as the water-vascular system bearing 4 madreporites found in this species does not seem to be causally related to its capacity of autotomous separation, yet it may favour the full accomplishment of regeneration following the self-separation.

Both the large and small arms appear in pairs. The larger pair always retain their physiological superiority over the smaller pair, and they dominate over the smaller arms in locomotion. The physiological superiority of the anterior end may become one of the incentives of self-division. The "physiological anterior" (COLE 1913) is apparently determined by the difference in size of the arms, and not by the position of the madreporites, contrary to COLE's view. The autotomy appears usually in young stages when the difference in size between large and small arms is considerable. In adult individuals in which the arms are of similar size, spontaneous separation rarely takes place.

(2) Regeneration of organs after division

When the disc is separated into semicircular halves, the injured end is closed by infolding of the tissues on the dorsal and lateral walls of the disc, as well as by the coming together of the rays adjacent to the open end (Figs. 3 B, C). In a few days, a newly formed tissue appears on the dorsal wall and also stumps of rays. Then the circumoral watervessel is completed close to the dorsal wall. Further regeneration proceeds rapidly around these newly formed tissues which are entirely pigmentless at first. The regenerative process seems especially vigorous in the oral and adoral regions. In nearly ten days a papillary bud appears on each side at the base of the regenerated part. This is the rudiment of the radial canal and radial nerve cord of the first ray (Fig. 2 D).

When the arm rudiments undergo further development, a part of the cardiac portion of the stomach enters the interior of the regenerated arms. This part forms the cardiac pouch which occupies a large part of the future ray cavity. The membraneous retractor muscles of the cardiac pouch are connected to the first ambulacral plate on each side.

With growth of the arm rudiments, a few calcareous plates appear at the base of each arm on the dorsal side. These plates increase rapidly in number (Fig. 3 A). When the radial vessels are elongated, the first pair of tube feet appear near the end of each vessel. At about the same time, some small spines arise on the calcareous plates which have become harder. These plates ultimately develop into the terminal plates which surround the azygous tentacles and the eye-spot, in connection with the terminal portion of the radial water-vessels. The other plates, except the terminal ones, are still very small, and carry no spines.

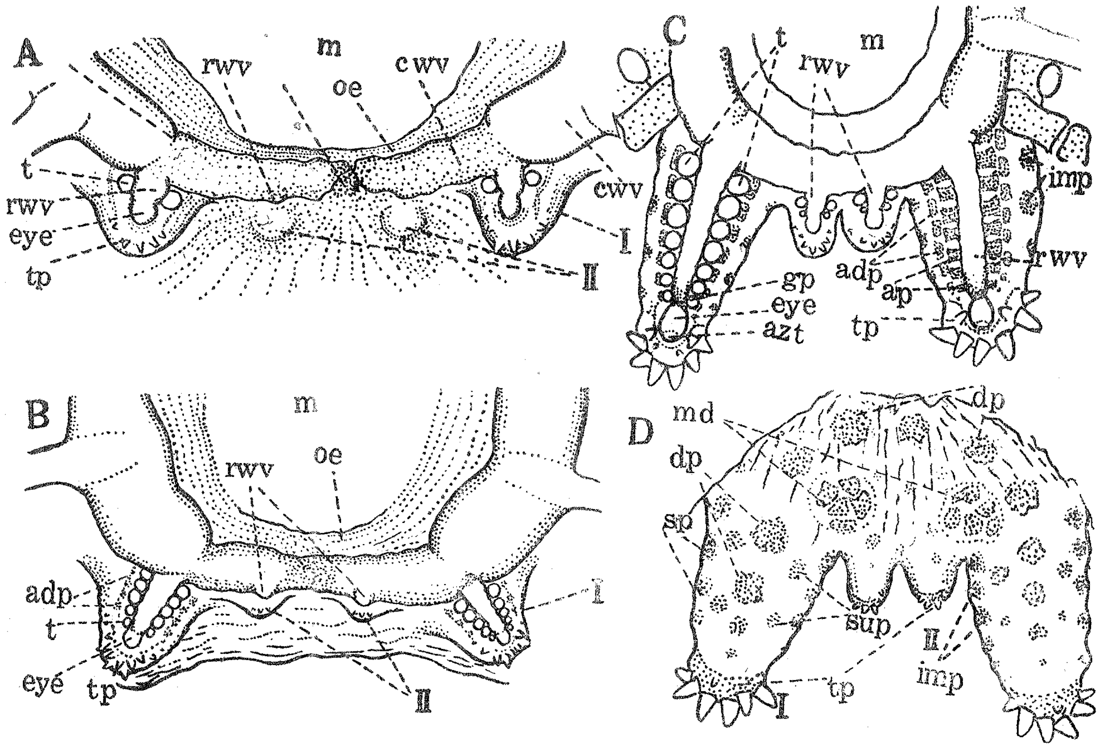

Fig. 3. More advanced stage of regeneration than in the foregoing figures.
(A) Actinal side, at the time when the rudiments of secondary rays (II) appear between primary rays (I).
(B) Actinal side, in more advanced stage.
(C) Actinal side, at the time when a series of tube feet begins to appear.
(D) Abactinal side, showing the formation of madreporites.
azt azygous tentacle; *eye* eye-spot; *imp* inferomarginal; *sup* superomarginal; *t* tube foot; *tp* terminal plate. Other legends as in the above figures.

The terminal portion of the radial water-vessel acquires a sac, which is simple at first, but soon undergoes differentiation. Its terminal end develops azygous tentacle, and the proximal broad part develops an eye-spot. The tube feet are at first simple diverticula arising from the wall of the radial water-vessel, but with the development of ambulacral plates, each diverticulum is devided by twisting into two parts, of which the one placed basally becomes an ampulla and the other at the distal end forms the tube foot. After the appearance of the first rays (I), two rudiments of stone-canals arise from the internal side of the circumoral vessel. These grow upwards until they reach the dorsal wall. When 3–5 pairs of tube feet appear in the first pair of ray (I), the second pair of rays (II) appear between the first pair, and grow in the same manner as the latter (Fig. 3 C). The regeneration of the circumoral vessel proceeds from both sides of the injured portion, and the two ends come to join in the middle.

In the stage when the first pair of rays (I) measure about 0.6 mm in length, the terminal plates bear 5–6 spines and the ambulacrals, adambulacrals and tube feet have heen formed in 5–7 segments. The dorsal plates appear a little later than these calcareous plates.

In a later stage when the I rays have 7–8 segments, and the II rays have 1–2 segments, an oesophagus has already been formed in the body (Figs. 3 C, D, *oe*). On the interradials between the I and II rays, two new madreporites are formed. Each of these madreporites consists of 5–7 calcareous plates of the same nature as the other dorsal plates, and is situated at the terminal end of the stone-canal rudiments (Fig. 3 D, *md*). In the interradial cavity of the II rays, a rudiment of the rectal sac appears from a part of the pyloric portion of the stomach, and opens to the anus. The remaining part of the pyloric portion fills up the proximal part of the ray cavity, and extends to the end of the rays (Fig. 4 A). For further development, a description will be given for each organ system.

Digestive system: The pyloric caecum soon furcates into two hollow canals at the position of the mesenteries placed on the median line of the ray cavity. The stalk of these branches remains as a common duct (Fig. 4 A, *pc*, *cd*). The branches which are originally simple canals, issue laterally two series of short lobular branchlets. Meanwhile, the median canal becomes flattened laterally (Fig. 4 C, *mc*). When the rectal sac grows larger and oval in outline, a transparent rounded anal rudiment appears of the dorsal wall, which opens externally soon afterwards. The elastic retractor muscles of the cardiac portion of the stomach gradually reach the 20th segments of the I rays.

Water-vascular system: The calcareous plates forming the madreporite become closely disposed together into a disc, and grow far more rapidly than any other dorsal plates. The tube-feet are arranged

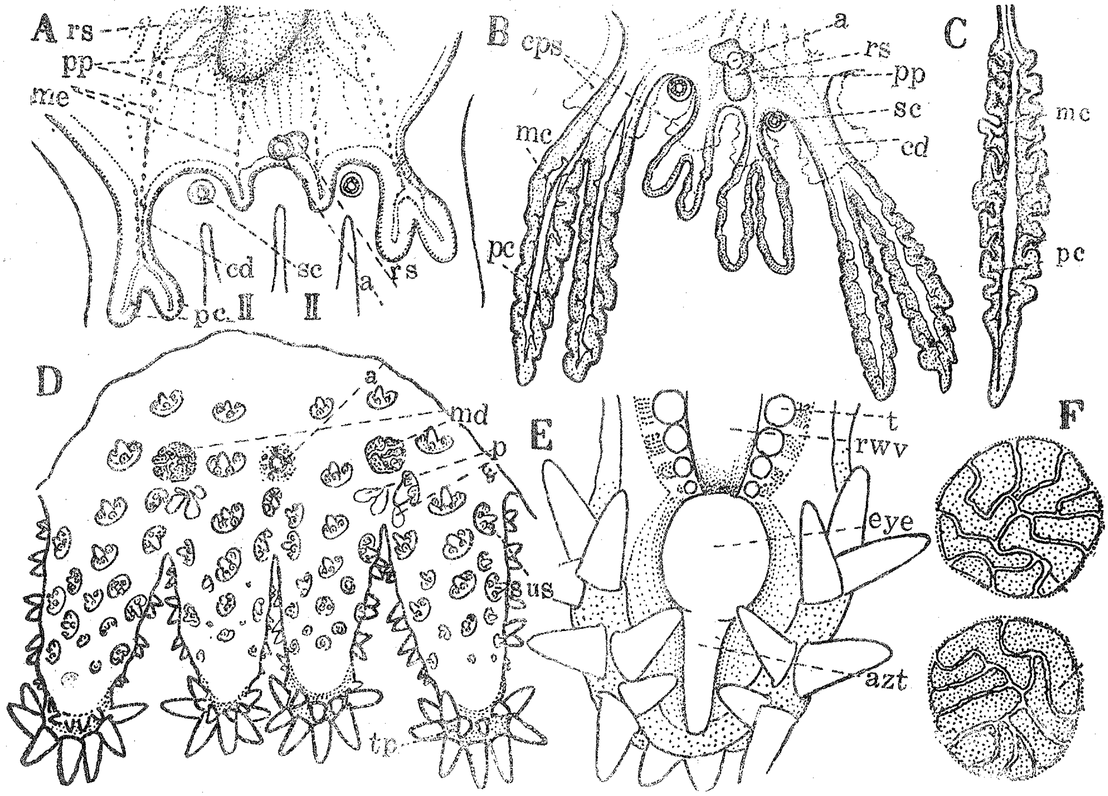

Fig. 4. (A) (B) (C) Development of digestive system, viewed from abactinal side.
(D) Development of terminal plates and madreporites.
(E) Terminal end of regenerated ray, highly magnified.
(F) Two madreporites, highly magnified.
mc median canal of pyloric caecum; *pc* pyloric caecum. Other legends as in the above figures.

in double alternating rows on each side of the radial vessel. They look at first rod-like, but become club-shaped by formation of terminal suckers. At the same time, the radial vessel becomes more flattened dorsoventrally. With the development of tube feet a series of ambulacral pores are formed, which are arranged in two straight lines, so that the arrangement shows a zigzag course as a whole. When there are 20 arm-segments, the area of the ambulacral plates becomes somewhat depressed, and the rudiments of TIEDEMANN's bodies are formed in each interradial, close to the circumoral water-vessels. The axial organ is also formed around the stone-canal. Prior to the full development of the dorsal plates, spines and crossed pedicellariae are formed on the dorsal disc. Some papulae and straight pedicellariae are also formed along the margin of the arms (Fig. 4 D).

The inferomarginals grow earlier than the superomarginals, the abactinals later (Figs. 3 D, 4 D, *imp*, *sup*). Each plate bears two spines, except the first inferomarginals, which have only one. After the formation of spines on these plates, the crossed pedicellariae appear on the marginal part. The first ambulacrals and adambulacrals become much larger than any other secondary plate, and form together the buccal ring around the mouth. Each plate of the other adambulacrals bears two spines of which one is directed inwards. With the growth of the animal, the pedicellariae and papulae on the dorsal surface increase in number. No gonad, however, appears in this stage.

REMARKS ON REGENERATION IN SOME OPHIURANS

Our knowledge of autotomous reproduction and regeneration in ophiurans is very meagre. The following is a brief summary of my observations on this process in four Japanese ophiurans, *Ophiothela danae* VERRILL, *Astroceras annulatum* MORTENSEN, *Ophiactis savignyi* MÜLLER et TROSCHEL and *Ophiactis modesta* BROCK. Some specimens of the first three species were obtained by myself from Kada and Seto, Wakayama Prefecture, and those of the last-named one were collected by Dr. S. MURAKAMI at Amakusa, Kumamoto Prefecture in Kyūsyū.

In all of these ophiurans, spontaneous division across the disc takes place normally throughout the whole life, and produces two halves of similar appearance. Of these species, *Ophiothela danae* is found often in great numbers, clinging to the stem of the gorgonian *Melitodes*, while the other species are common under stones or among seaweeds in the intertidal zone. All specimens of these ophiurans hitherto obtained have 6 rays as far as my observation goes. These ophiurans have no definite line of fission, and self-division may take place across the disc along any axis. Therefore, the detached half resulting from fission has always 3 rays. The unpaired dorsocentral and oral shields belong to either one of the detached halves. Such indefiniteness in the axis of fission is probably a characteristic of ophiurans, correlated with the absence of madreporite and anus, and also with the true radial symmetry of the whole structure.

These ophiurans seem to divide themselves several times in life, and individuals which have apparently regenerated four times are sometimes observed. It is easy to tell how many times the division has taken place, by comparing the length of arms, ray segments, radial shields and ambulascral plates of each individual. Thus these ophiurans propagate asexually as well as sexually. In all cases, three new rays appear to regenerate from one half of a spontaneously divided disc, Of the regenerated rays, the two lateral ones grow faster than the middle one.

CLARK (1911) has noted in *Astroceras pergamena* having 6 rays that "None of the specimens reveals how the 6-armed young becomes the symmetrical 5-armed adult but in two specimens the middle arm of the smaller trio is distinctly smaller than its two neighbours, and it may be that this middle arm is ultimately resolved or lost by autotomy." MATSUMOTO (1917) considers, when describing *Ophiactis savignyi*, that "The smaller size of the arm in question is perhaps due to its unfavourable position with regards to the supply of nutrition."

According to my observations on these species, however, the smaller size of the regenerated middle arm seems to be due merely to retarded development, contrary to the views of these authors.

The usual regeneration in these ophiurans, as far as I have observed, proceeds in the following order:—dorsals, first adambulacrals, terminals, laterals, interradials, second adambulacrals, dorsal arm plates, tentacle scales, dental plates and teeth, then only in later stages the radial shields and oral shields appear.

SUMMARY

1. *Coscinasterias acutispina* is usually 8-rayed, and exceptionally 6-, 7-, or 9-rayed.

2. Autotomous separation takes place until its gonads become ripe. This occurs especially frequently in juvenile stages in the season from early spring to autumn. The fission always follows the axis between the pair of anal openings on the disc.

3. Different individuals may have rays ranging from 4 to 9 in number, but 8 is apparently the normal number for this species. All the newly regenerated individuals are provided with 4 madreporites, 2 anal openings, 2 rectums carrying a few rectal sacs and 12–16 TIEDEMANN's bodies.

4. The large and small rays are always found in pairs, the large rays retain physiological superiority over smaller ones, and lead them in locomotion. COLE's "physiological anterior end" is mainly determined by the size of rays instead of the position of madreporites.

5. An outline of the regenerating process of internal organs in *C. acutispina* following the self-division is given.

6. A brief description of the regeneration process following spontaneous division in four ophiurans, *Asteroceras annulatum*, *Ophiactis modesta*. *Ophiactis savignyi* and *Ophiothela danae* is also given.

7. In these sea-stars and ophiurans, autotomy is used as a method of asxual propagation, and it belongs to the normal and physiological processes in these forms.

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