Marine Biology 5, 245—255 (1970)

Anthopleura stellula (Actiniaria, Actiniidae) and its reproduction by transverse fission

H. Schmidt

1. Zoologisches Institut der Justus Liebig-Universität; Gießen, Germany (FRG)

Abstract

Anthopleura stellula Ehrenberg 1834, a sea anemone from Eilat, Red Sea, is redescribed in detail and shown to be synonymous with *Anthopleura elatensis* England 1969. Asexual reproduction by autonomous transverse fission has been observed over a period of 8 months in *A. stellula* maintained in Mediterranean seawater (37.0 to $37.5^{0}/_{00}$ S). Fission always occurs at the same level, below the pharynx, and is followed by an asynchronous regenerative process beginning at the initial point of cleavage. The distal portion of a bisected anemone regenerates a new pedal disc, while pharyngeal formation is induced by the mesenteries of the first-budding directive tentacle in the proximal half; the original oral disc pattern is restored. After regeneration has been completed, the newly formed anemone can similarly engage in transverse fission. The fission process spreads infectiously. If *Bunodactis verrucosa*, a Mediterranean anemone, is maintained in the same aquarium with *A. stellula* then the beginnings of fission followed by dedifferentiation are observed in the Mediterranean species, which does not ordinarily reproduce asexually. *A. stellula* still exhibits its induced ability to divide transversly if transferred into fresh Mediterranean seawater. When these specimens are exposed to low temperatures (12° to 13 °C) for 2 months, fission activity still remains, but for only one division; there is no further fission, and no regeneration of a pedal disc. Abrupt increases in salinity to the salinity level of the Red Sea (40.0 to $41.0^{0}/_{00}$) interrupt the fission activity and pedal disc regeneration. *A. stellula* incised at the level where fission usually occurs promptly undergo transverse fission if cultured with other dividing anemones. However, the other anemones merely exhibit wound healing if the salinity is high. It is thought that fission and regeneration are induced by stimulating substances produced by the anemones during low salinity conditions and that the stimulating substances accumulate in the aquarium water.

Materials and methods

Twenty two specimens of *Anthopleura stellula* were maintained in an aquarium filled with 40 l Mediterranean water, and exposed to north-westerly daylight. Salinity was maintained between 37.0 and $37.5^{0}/_{00}$, temperature between 22° and 24 °C. The water was filtered continuously using an "Eheim" filtration system; about half the water was replaced bi-monthly. The sea anemones were fed weekly with small pieces of *Mytilus galloprovincialis* and daily with fresh plankton or *Artemia salina* nauplii.

Higher salinities were obtained by addition of "Instant Ocean" salts (Aquarium System, Inc. Wickliffe, Ohio, USA).

Serial sections were made of 8 specimens fixed at Eilat (Red Sea) and 22 dividing or regenerating specimens. The specimens were narcotized with 7.5% magnesium chloride and fixed in Bouin-Hollande solution. The sections were cut at 7 μ and stained by a variety of techniques.

Best results were obtained by the following staining procedure: Sections were stained in Mayer's haemalum solution for 5 to 6 min, then counterstained in an eosin-orange G solution for 2 to 3 min [25 ml of a 1% orange G stock solution mixed with 30 ml of a 0.1% eosin (water soluble) stock solution and 3 to 4 drops of glacial acetic acid made up to 250 ml by the addition of distilled water]. The collagen was then mordanted in 5% phosphotungstic acid solution for 1 min and stained in a 0.5% aniline blue solution mixed with 0.25% glacial acetic acid for 2.5 min. The sections were rapidly rinsed in 90% alcohol and dehydrated in absolute alcohol. This method was supplemented by the use of Mayer's haemalum and eosin and Heidenhain's iron haematoxylin.

Taxonomy

Introduction

During the MAMBO[1]-course, in the winter of 1965/1966, I observed and collected specimens of *Anthopleura stellula* from the beach at Eilat, Red Sea. I transported both fixed and living specimens of this anemone to Naples (Italy) in order to study its reproductive processes.

This species has been described using specimens collected from the Red Sea (Ehrenberg, 1834; Klunzinger, 1877) and from the coast of Zanzibar (Carlgren, 1900). The first record at Eilat was made by Lipkin (Safriel and Lipkin, 1964).

This species was recently described incorrectly under the name of *Anthopleura elatensis* n. sp. by England (1969) on the basis of formalin-preserved specimens collected at Eilat. My observations on specimens from the same locality, and comparisons with the material of Ehrenberg (1834) and Carlgren

---

[1] Mediterranean Association of Marine Biology and Oceanology.

(1900), confirm that the species from Eilat is *Anthopleura stellula* Ehrenberg 1834. Because this species is still poorly known, I have composed the following detailed description using living specimens.

Description

Genus *Anthopleura* Duchassing and Michelotti, 1861

Actiniidae with well-developed base. Column with verrucae, arranged in more or less distinct vertical rows and with acrorhagi which may be situated on the parapet or in the fosse. Sphincter weak or strong, circumscript or circumscript diffuse. Tentacles simple, hexamerously or irregularly arranged; their longitudinal muscles are principally ectodermal. Perfect mesenteries are numerous. Retractors are distinct, either diffuse, or sharply limited. Gonads appear on the older mesenteries. From the second cycle onward, the mesenteries grow from the proximal end toward the distal end, and may therefore sometimes exceed the tentacles in number (Stephenson, 1935).

Anthopleura stellula (Ehrenberg, 1834)

Synonyms: *Actinia Isacmaea stellula* H. et E. Ehrenberg, 1834. *Actinia Isacmaea - stellula* Ehrenberg. *Monostephanus* (Brandt, 1835). *Isacmaea stellula* Ehrenberg (Milne Edwards, 1857). *Bunodes stellula* Ehrenberg (Klunzinger, 1877; Andres, 1884). *Anthopleura stellula* Ehrenberg (Carlgren, 1900; Carlgren, 1949). *Anthopleura elatensis* England, 1969.

Description of an individual (Fig. 2a)

Base: pale dirty yellow, larger than the column, limbus notched by the mesenterial insertions, moderately adherent, mesenterial insertions marked as pale brownish, radial stripes.

Column: pale yellow, becoming grayish-yellow towards the margin, skin smooth, slightly corrugated, mesenterial insertions marked as brown lines, 40 in number; endocoels bearing adhesive verrucae, variable in number, on the upper part of the column; the exocoels are smaller than the endocoels and have no verrucae; margin with 20 whitish endocoelic acrorhagi; the fosse is indistinct during expansion.

Tentacles: 40 (6 + 6 + 12 + 16), conical, the inner tentacles longer than the outer ones; largest are as long as diameter of oral disc; hyalin, translucent, their surface slightly greenish-brown or gray, with irregular hyalin spots.

Oral disc: 5 radii belonging to the primary and second cycles (including the directive radius) are as white as the bases of the corresponding tentacles; the outer halves of the radii of the tertiary and quarternary cycles are greenish-brown except one tertiary radius, which is colored over its whole length. The base of the opposite directive tentacle and the corresponding radius are not as highly pigmented, and are clearer than the rest of the greenish-brown radii, which have some hyalin spots on their midlines.

Variation. The 62 collected specimens differed widely as to size. Therefore, the number of longitudinal stripes, acrorhagi and tentacles are variable. The tentacles are arranged hexamerously; however, the fourth and sometimes the third cycles are incomplete. The largest individual with 45 tentacles was 7.5 cm high when fully expanded (at night), with a scapus diameter of 1.2 cm, a pedal disc diameter of 1.7 cm, and an oral disc diameter of 2.2 cm. Most specimens were smaller than this, having occasional acrorhagi only on the first and second cycle. The acrorhagi are conical and very prominent. The verrucae (warts) are sometimes very indistinct (Fig. 2 a). All specimens had 2 siphonoglyphes and 2 directive tentacles.

There are 3 true varieties of oral disc color patterns: (a) the one already described is limited to males and has been found most frequently; (b) females lack the white sector and have only 2 clear, less deeply colored, sometimes whitish, directive radii; (c) in a third variety, all radii of the third cycle are clear and less deeply colored (this variety could not be sexed since collection was out of season).

Anatomy. The number of mesenteries (average 43) usually exceeds the number of tentacles (average 32); mesenteries are always paired, and hexamerously arranged in 3 cycles in large specimens containing 48 in number. The first, second, and part of the third cycles have perfect mesenteries; some pairs of the third cycle only partly reach the edge of the sphincter, since the mesenteries grow from the proximal end upward. Oral and marginal stoma are present. Mesenteries of the first 2 cycles and, rarely, of the third cycle, are provided with filaments; these are situated more proximally on the first cycle than on the other 2 cycles. The septal filaments occur in an extreme proximal position on the directives. Gonads only appear proximal to the ciliated tracts. They are present on the first cycle (usually also on the directives) and on some mesenteries of the second cycle. In large specimens, some mesenteries of the third cycle have filaments in their distalmost regions; however, because these filaments are provided with ciliated tracts, they permanently lack gonads. Most individuals have no filaments on the third cycle. The pharynx contains longitudinal ridges and 2 distinct siphonoglyphes. The retractors are diffuse circumscript (England, 1969, Fig. 2c) or diffuse; the parietobasilar muscles form distinct folds, and the basilar muscles are weak. The sphincter is highly variable not only between specimens (England, 1969), but also within the same specimen. The sphincter is diffuse or circumscript-diffuse. The warts can become calyx-like, and may take up gravel or other foreign bodies.

Cnidom. (Fig. 1) Spirocysts, isorhizic and anisorhizic haplonemes, b-rhabdoides (2 variations), p-rhabdoides A (Schmidt, 1969).

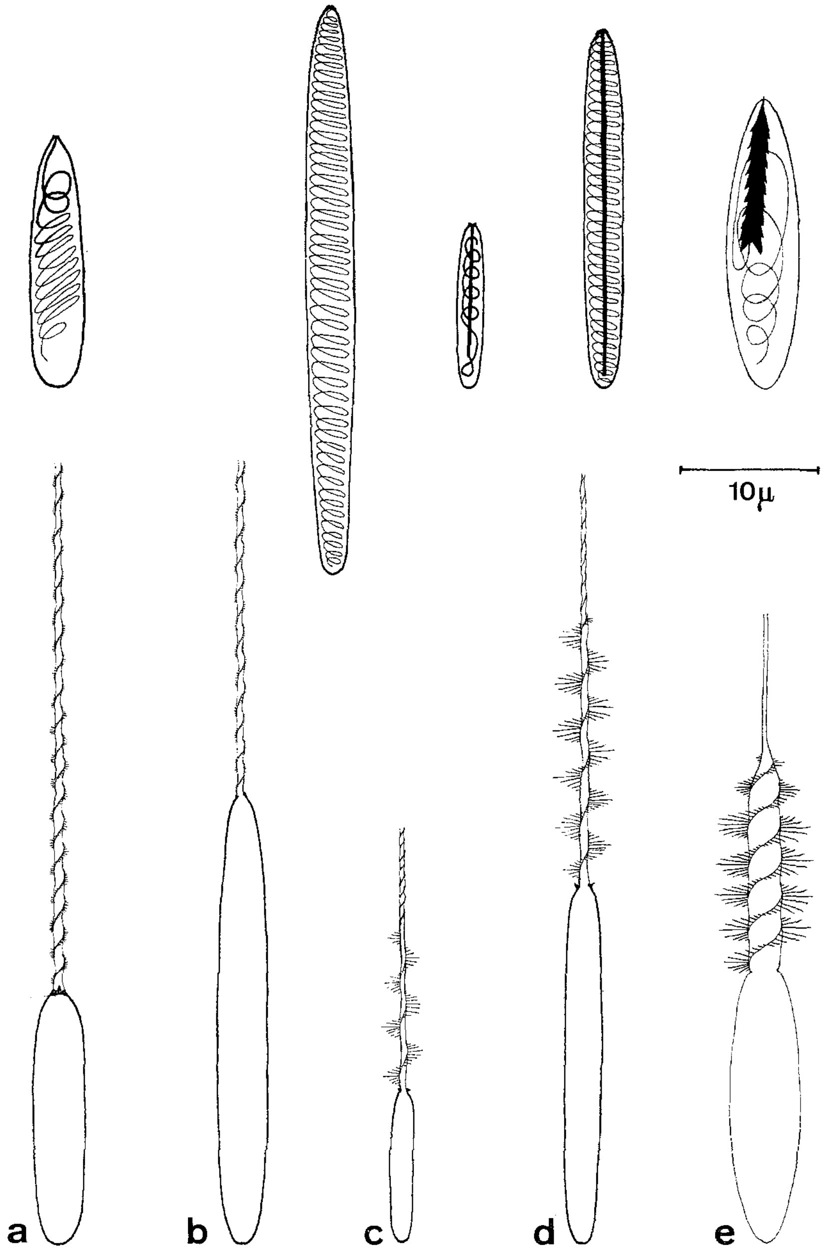

Fig. 1. Nematocysts of *Anthopleura stellula*. (a) Anisorhizic haploneme (thread 6 × capsule) of the column; (b) isorhizic haploneme (thread 16 × capsule) of the acrorhagi; (c) b-rhabdoide (thread 4 to 6 × shaft) of the filaments; (d) b-rhabdoide (thread more than 10 × shaft) of the pharynx, also representative of the tentacles and filaments; they are more club-like in the latter; (e) p-rhabdoide A of the filaments, also representative of the pharynx

Size and distribution of nematocysts

Column:

b-rhabdoides 16.5(9.8—19.5) × 2.5(2.0—3.2)μ,
anisorhizic haplonemes 18.8(14.3—24.7) × 4.0(2.6—4.6)μ.

Acrorhagi:

isorhizic haplonemes 38.0(32.2—42.2) × 4.4(2.6—5.2)μ.

Tentacles:

spirocysts 19.1(13.0—21.4) × 3.4(2.6—3.9)μ.
b-rhabdoides 18.2(16.2—20.8) × 2.6(2.1—3.3)μ.

Pharynx:

b-rhabdoides 24.8(22.1—27.3 × 3.1(2.5—3.9)μ.
p-rhabdoides A 20.7(18.0—22.0) × 4.3(3.5—5.5)μ.

Filaments:

b-rhabdoides 12.4(9.8—15.6) × 2.2(1.6—2.6)μ.
b-rhabdoides 27.0(24.7—28.6) × 4.3(3.2—5.9)μ.
p-rhabdoides A 20.2(17.6—22.1) × 4.9(3.3—6.5)μ.

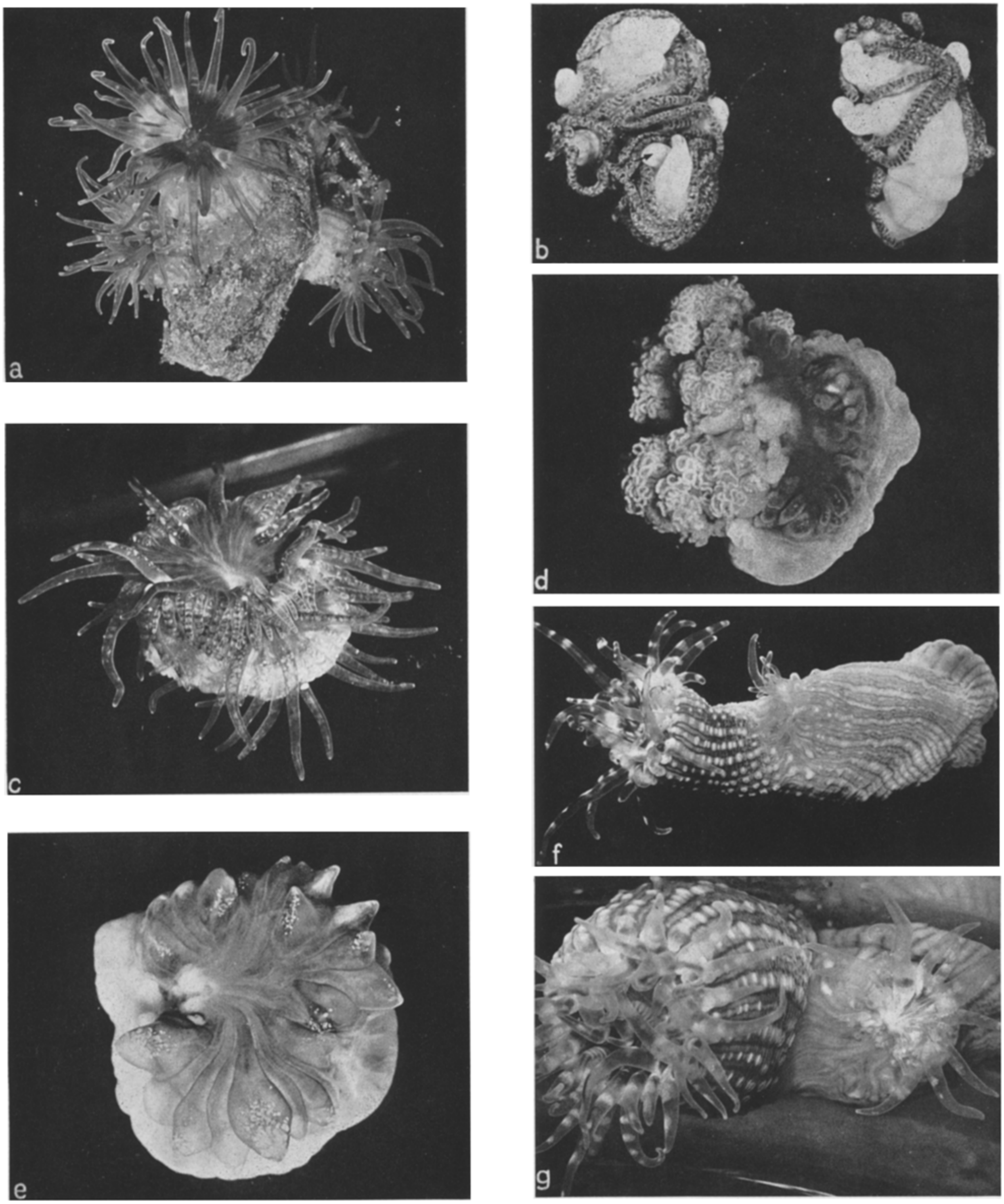

Fig. 2. (a) *Anthopleura stellula*, males collected at Eilat, Red Sea, 1.8 ×; (b) *Staurosoma parasiticum*, dissected from its capsule, entwined by egg tubes; dwarf male (arrow), 8 ×; (c) *Bunodactis verrucosa*, exposed 43 days to seawater containing dividing *Anthopleura stellula* specimens. This anemone performed half a transverse fission and regenerated half another tentacle crown, 1.3 ×; (d) *Bunodactis verrucosa*, exposed 56 days to seawater containing dividing *Anthopleura stellula* specimens; warts and tentacles have been absorbed, 1.8 ×; (e) *Bunodactis verrucosa*, same individual as in D, 13 days after removal from the medium containing dividing *Anthopleura stellula* specimens: tentacle stumps begin to grow, note the open pharynx, 2 ×; (f) *Anthopleura stellula*, 19 days after interruption of transverse fission by raising the salinity; new tentacles, acrorhagi and warts develop on about one half of the proximal part, 1.8 ×; (g) *Anthopleura stellula*, 29 days after interruption of fission by raising the salinity; half an oral disc with half a pharynx have formed. Note undifferentiated tissue between new oral disc and distal part, 2 ×

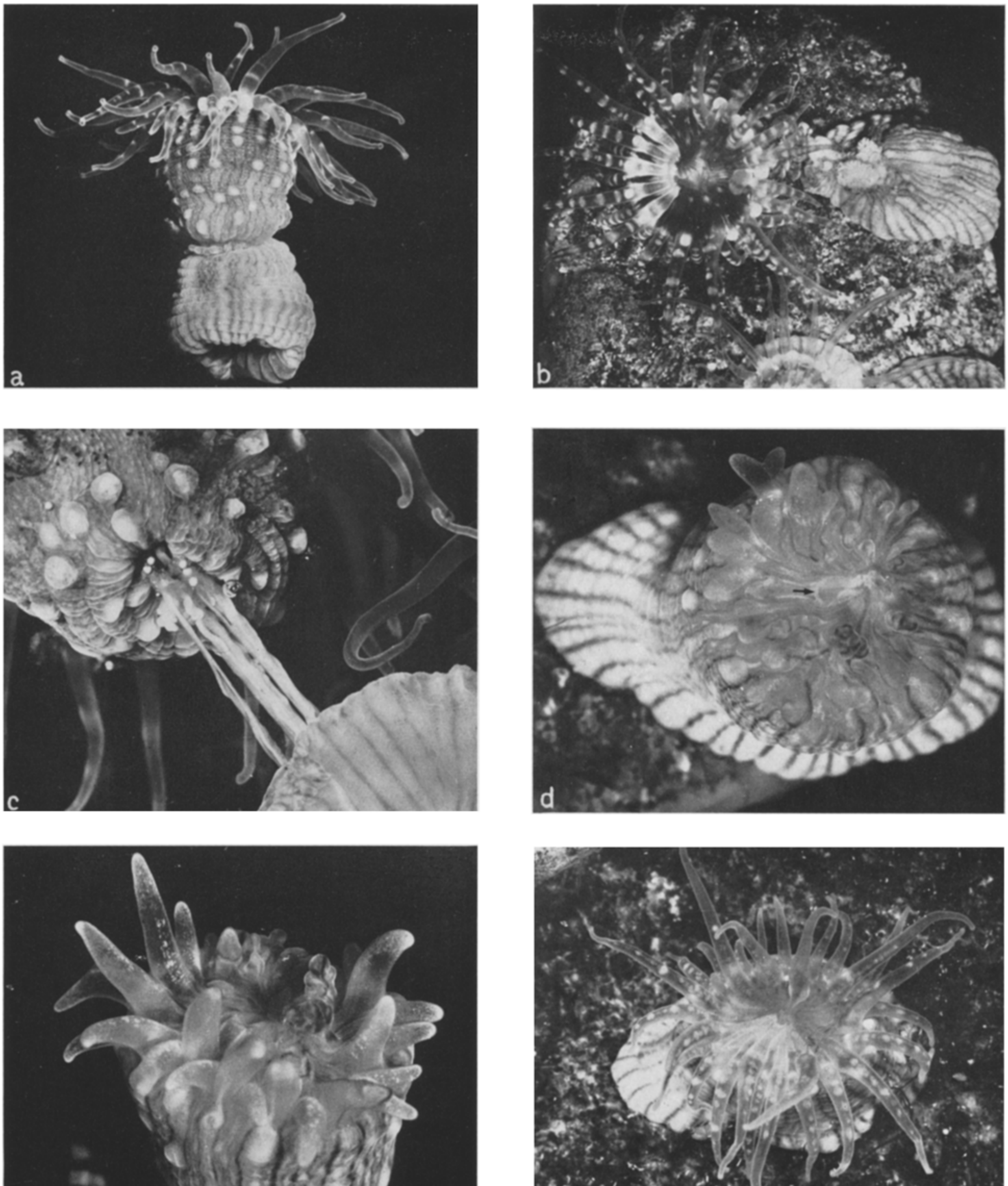

Fig. 3. *Anthopleura stellula.* (a) beginning transverse fission, ectodermal processes are split off by gastrodermal constriction, 2 ×; (b) just after transverse fission, proximal part creeping away, 2 ×; (c) just after transverse fission, photo taken from below. The septal filaments are still connected; note adhesion by warts of the distal part, 6 ×; (d) proximal part of specimen shown in B, 7 days after fission; note pharynx formation on one side only and directive radius (marked by arrow) pointing at the new oral border, 5.5 ×; (e) proximal part of the specimen shown in B, 10 days after transverse fission; tentacle crown not yet completed and ectodermal stumps still present, 5 ×; (f) proximal part of the specimen shown in B, 16 days after transverse fission; note restoration of original oral disc pattern (compare with B). Irregular dark lines are remaining ectodermal stumps which are being absorbed, 3 ×

Ecology. *Anthopleura stellula* were found on the stony beach at Eilat in shallow water (20 to 50 cm) under flat stones. The anemones always occurred in small groups of 3 or 5, but sometimes more individuals adhered to a small stone (Fig. 2a). A cavity in an old concrete-filled barrel standing in shallow water was crowded with specimens. The colors of the anemones are not very pronounced, as a result of their shady habitats. However, after exposure to full daylight for several weeks in the aquarium, the anemones develop bright colors (compare Fig. 2a, f, g; Fig. 3a, b). The specimens were arranged in small groups in Boveri-bowls in the aquarium. After a short period, all anemones crept over the edges of the bowls and settled underneath the bowls, demonstrating their typical cryptophilic habits. They were still continuously exposed to daylight, despite this position. Under these conditions, the hyalin radii on the oral disc and the hyalin spots on the tentacles became whitish, the warts and the acrorhagi became very white and the longitudinal striations turned a deep greenish-brown. Simultaneously, the distal part of the scapus became reddish. The species is able to survive at 12° to 13 °C.

Reproduction. *Anthopleura stellula* reproduces both asexually and sexually while maintained in the aquarium. The species is oviparous; eggs are 200 μ in diameter and have 18.6 μ long spines; sperms are about 60 μ long with a rounded head (6.5 μ). *Anthopleura stellula* is seasonally fertile. The specimens fixed at Eilat in January contained only 2 females with eggs. The other specimens were without gonads at this time. All anemones in the aquarium developed fully ripened gonads (Fig. 3b) during the summer, when the temperature of the surface water rises in the gulf of Acquaba (OREN, 1962).

Parasites. Five specimens formed capsules of about 0.5 cm diameter in a single mesentery (in 2 mesenteries in one case); the capsules were inhabitated by the parasitic copepod *Staurosoma parasiticum* WILL (1844). One anemone lodged 3 copepods, each within a separate capsule. The parasites were entwined by long tubes filled with eggs (Fig. 2b). Further details are described by LAUBIER and SCHMIDT (In press).

Distribution. Living *Anthopleura stellula* were collected by EHRENBERG (1834) at Massaua and by KLUNZINGER (1877) at Koseir. STUHLMANN found this species outside the Red Sea on the coast of Zanzibar (CARLGREN, 1900). The present known range of this species extends as far as Eilat, in the north of the Red Sea.

Discussion of taxonomy

The specimens of *Anthopleura stellula* collected at Eilat have been compared with the description of EHRENBERG (1834), KLUNZINGER (1877) and CARLGREN (1900), and with the type specimens of EHRENBERG[1] and CARLGREN[2]. The observations presented in this report are substantiated by the above material, except for CARLGREN's statements on the sphincter and the cnidom (compare ENGLAND, 1969). Contrary to CARLGREN's report, the sphincter of the specimen he examined is not strong circumscript but diffuse circumscript. Moreover, CARLGREN has overlooked the p-rhabdoides A which occur also in the pharynx of his specimen.

ENGLAND (1969) stated that a single ovary exists on one third cycle mesentery. However, there are no gonads on the third cycle at all (compare p. 246). ENGLAND has probably overlooked the possibility that egg-bearing parasitic copepods may inhabit a single mesentery, thus giving the appearance of gonadal development. The sphincters drawn by ENGLAND (1969, Fig. 2a, b) are typical for this species. However, they are not "diffuse with a tendency to be restricted", but diffuse or diffuse circumscript, as compared with the figures and the terminology given by STEPHENSON (1928) and CARLGREN (1949).

ENGLAND's (1969) statement on the cnidom, demonstrates that it is not possible to make a useful species' description solely on the basis of fixed material. His drawings (ENGLAND, 1969, Fig. 3) and measurements of the nematocysts, clearly show that he has confused unripe and ripe nematocysts; only the latter are of taxonomic value. The unripe nematocysts of sea anemones are larger than the ripe ones (SCHMIDT, 1969).

Anthopleura thallia, a sea anemone very similar to *A. stellula*, inhabits certain regions of the British coast; it has not been taken into consideration by ENGLAND (1969). *A. thallia* has an irregular arrangement of tentacles and mesenteries and reproduces by longitudinal fission. It is found under stones in shallow water (STEPHENSON, 1935) like *Anthopleura stellula* at Eilat and Koseir (KLUNZINGER, 1877). In regard to *Anthopleura thallia*, STEPHENSON (1935) reports "I have seen anemones in the Cape Peninsula and also on the Great Barrier Reef, which must be very closely related to *A. thallia*; and I suspect that some at least of them are identical with it, and that its range may, in reality, be very wide". The observations of STEPHENSON suggest that *A. stellula* is probably spread throughout the Indo-Pacific Ocean and occurs even in the Atlantic Ocean.

Reproduction by transverse fission

Introduction

Asexual reproduction is common in several actinians and other coelenterates, but spontaneous

---

[1] *A.* (*Isacmaea*) *stellula* H. & E., Massaua 166 H. & E.: Inst. Spez. Zool. u. Zool. Mus. Humboldt-Universität, Berlin, GDR.

[2] *Bunodes stellula* Stuhlmann Sansibar C 2635: Zool. Staatsinst. u. Zool. Mus. Hamburg, FRG.

transverse fission has seldom been observed in these animals.

One actinian species which does reproduce by this method is *Gonactinia prolifera* SARS (1835), BLOCHMANN and HILGER (1888), PROUHO (1891), KERB (1913) and CARLGREN (1929). The transverse fission of *G. prolifera* is considered as a normal but unique mode of asexual reproduction of a "primitive" anemone. Very little is known about transverse fission among other species of sea anemones. The only authentic report is more than 100 years old; BRODERICK (1860) observed spontaneous transverse fission in 3 specimens of *Aiptasia couchii*. HOLDWORTH (1860) and ANDRES (1882) hypothesized that transverse fission was the mechanism responsible for the doubling of regenerating specimens of *Aiptasia couchii* and *Aiptasia lacerata*. Later, STEPHENSON (1935) mentioned that transverse fission exists in *Aiptasia couchii* (recorded by EVANS; STEPHENSON, 1935).

In summary, detailed studies of transverse fission have only been conducted for *Gonactinia prolifera*, and it would therefore be instructive to study this process in other actinians and to compare the results with those of the classical studies. A further purpose of the experiments presented in this report is to determine the morphological events which accompany transverse fission and regeneration in *Anthopleura stellula*. In addition, the conditions necessary for the occurrence of transverse fission and regeneration in this species will be ascertained, and the circumstances responsible for the induction of fission in other non-dividing species will also be determined.

Observations

Fission

After maintaining *Anthopleura stellula* in the aquarium for 5 months under Mediterranean salinity conditions, the first fission was observed in a constricted anemone. Tentacle-like processes projected from the fissure giving the impression that this anemone divides by paratomy (Fig. 3a). These processes are of ectodermal and mesogloeal origin and are divided into longitudinal ridges by the marked contraction of the entodermal circular muscle layer. Fission begins on one side of the column and progresses to the opposite side just below the pharynx until the anemone divides. The larger distal part is highly colored, and provided with warts which are absent on the column of the smaller proximal part (Fig. 2a; Fig. 3f). At the end of the fission process, the distal part rotates about the last connection to the proximal segment of the column and settles close to it (Fig. 3b). The mesenteries separate more passively. The remaining connections between the distal and proximal parts are the filaments because they are much longer than the mesenteries (Fig. 3c). The fission process is completed within 1 h, but the filaments may still, in some cases, connect the segments up to 48 h later. The proximal part glides away from the distal part just after columnar separation is completed, thus tearing the connecting filaments (Fig. 3b, c). The distal part attaches to the substrate by means of its warts and the infolded column, and is therefore unable to creep away.

Regeneration

After 4 days, white tentacle buds arise from the margin of a new oral disc formed by the upper columnar region of the proximal segment. The ectodermal processes formed during fission are situated centrally to the budding tentacles (Fig. 3d, e). These stumps are slowly absorbed into, or detach from, the new oral disc during subsequent regenerative process (Fig. 3f).

The first tentacle appears at the initial point of fissure, and the new warts also form on this side of the upper column. The wound on the distal segment is, at this time, covered with a mucuous film. Tentacle buds occupy about half the oral disc 6 to 8 days after fission, and it is now that the new pharynx forms. At first, the directive mesenteries fuse with the edge of the wound. The pharynx growth begins in the upper region of one of the directive mesenteries (corresponding to the first budding directive tentacle) originating from the endodermal layers. The 2 mesenteries are thus connected by the presumptive siphonoglyph tissue in their distalmost regions. The pharynx then grows obliquely towards the pedal end, and towards the opposite side (Fig. 3d). Each perfect mesentery pair takes part in pharyngeal formation when the corresponding tentacle bud appears. The smaller mesenteries fuse with the pharynx via their newly formed septal filaments provided with ciliated tracts. The uncolored pharyngeal tissue forms a distinct edge, fusing with the margin of the wound (Fig. 3d). At this time, the distal part of the divided anemone has completely healed and has regenerated a new, delicate, pedal disc which is scarcely able to adhere to the substratum. On the seventh day one can observe several cycles of tentacle buds on one side of the new oral disc. The radii of the first cycle become colored on the oral disc surface, while on the endocoels of the first cycle acrorhagi buds appear (Fig. 3d).

With the completion of the tentacle crown between the eleventh and the thirteenth day after fission, the pharynx surrounds the opening in the oral disc, which now becomes a functional mouth enabling the anemone to feed upon small pieces of *Mytilus galloprovincialis*. From the twelfth day onward, mesenteries grow on the new pedal disc. The warts disappear from the proximal area of the former distal part, while the anemone originating from the proximal part develops the same oral disc pattern exhibited by the distal part (compare Fig. 3b and f).

It is also noteworthy that the fission process begins quite sporadically, but soon spreads infectiously to all individuals in the aquarium. The rate of fission increases so rapidly with time that fully regenerated anemones, derived from the distal and proximal portions of a single sea anemone, can likewise be observed in the process of division.

The original stock of 22 sea anemones acquired at Eilat gave rise to 147 individuals upon completion of this investigation. Although new fissures were sometimes observed before the entire disappearance of the warts on the lower column of the distal part, there was usually no fission before regeneration was completed. All anemones underwent transverse fission without exception, so that the size of the individuals in the aquarium progressively diminished. These smaller anemones exhibited fission much more frequently than larger specimens.

This summary of transverse fission and regeneration has been compiled from many detailed observations made within an 8 month period. Other details of this study will be published later.

Experiments

Experiment 1. 12 non-dividing specimens which were incised below the pharynx promptly performed transverse fission and regeneration when maintained in an aquarium containing dividing anemones.

Experiment 2. 10 non-dividing specimens were transferred to another aquarium filled with fresh Mediterranean seawater. The first fission was observed after 11 days; the division frequency increased with the passage of time.

Experiment 3. 3 specimens which were about to divide were placed in Mediterranean seawater at 12° to 13 °C for 2 months. This prevented fission; 1 anemone died. The 2 remaining anemones were then transferred to Mediterranean seawater of their original habitat temperature (22° to 24 °C) and both divided 3 weeks later. However, the resulting fragments exhibited no further fission; the proximal parts regenerated and the distal parts died 23 to 28 days after this terminal fission.

Experiment 4. 3 specimens of each of the following Mediterranean species were placed into an aquarium containing dividing *Anthopleura stellula: Aiptasia couchii* (a sea anemone in which transverse fission has been reported), *Anthopleura rubripunctata* (whose juvenile stages, *Bunodactis sabelloides*, are able to reproduce by longitudinal fission), and *Bunodactis verrucosa* (which never reproduces asexually). *Aiptasia couchii* and *Anthopleura rubripunctata* did not exhibit fission, but significant structural alterations occurred in *Bunodactis verrucosa*. After 27 to 31 days, 2 specimens of *B. verrucosa* formed a transverse slit in the upper parts of their columns. However, fissure did not completely transect these animals, and 2 weeks later new tentacles grew from the proximal border of the opening on only one side of the column (Fig. 2c). An unusual type of dedifferentiation then took place; the anemones lost all their warts and their tentacles became considerably shortened. The third animal soon developed in this way. After 56 days, all 3 anemones were in very poor condition: the filaments had been shed and a part of the column, including a portion of the oral disc and a part of the pharynx, were open (Fig. 2d, e). Eventually, 1 specimen of *Bunodactis verrucosa* died; the specimens of the other species were, however, still in good condition. The surviving individuals of *B. verrucosa* recovered after being placed in another aquarium. It took nearly 3 months for these anemones to acquire normal tentacles and warts.

Experiment 5. Finally, the salinity was increased to about that of the Red Sea (between 40.0 and $41.0^0/_{00}$) by addition of sea salts. This treatment immediately interrupted the process of transverse fission in *Anthopleura stellula*. Dividing anemones lost their constriction and regenerated tentacles on the proximal border of the wound at a slightly retarded rate (Fig. 2f). Other dividing specimens lost the constriction without forming tentacles. No transverse fission was exhibited in any of these cases. Furthermore, 9 out of 10 anemones, which were slightly incised transversely at the level where fission usually occurs, healed within a few days instead of forming tentacles; only 1 anemone reacted like a dividing specimen. In cases where tentacle growth did occur, half an oral disc and part of a pharynx formed after 4 weeks. The opposite half of the column consisted of undifferentiated tissue, being neither oral disc nor pedal disc (Fig. 2g). The distal parts of such individuals detached from the proximal parts and died. In one case this happened after 80 days; the proximal part then regenerated normally.

Discussion and conclusions

Transverse fission in *Anthopleura stellula* is quite different from the division process in *Gonactinia prolifera*. In contrast to *A. stellula*, *G. prolifera* grows new tentacles before fission occurs (paratomy). Furthermore, additional fissures have been observed in the distal segment before it has separated from the proximal part (Prouho, 1891; Kerb, 1913). Fission in *A. stellula* is always followed by regeneration (architomy), and repeated fission has never been observed before regeneration is complete.

Several authors working on transversely cut actinians or cerianthids, found an oral-aboral gradient in tentacle regeneration; the new tentacles grew more quickly if the amputation level was closer to the oral disc than the pedal disc (Child, 1903b; Hazen, 1903; Cotronei, 1924). This gradient is distinctly demonstrated in obliquely amputated anemones (Moszkowski, 1907; Abeloos, 1955; Singer and Palmer, 1969).

In *Anthopleura stellula*, fission always occurs at about the same transverse level on the body column. However, there is a delay of about 8 days between the appearance of the first budding tentacle and the initiation of tentacular growth on the opposite side where the column ruptures last. This observation suggests that there is a horizontal gradient in transverse fission and regeneration, instead of the abovementioned gradients.

The level at which transverse fission is performed is always the same, just below the pharynx (indicated by the ends of the longitudinal rows of warts). This zone of fission may be functionally similar to the "budding region" of *Hydra* species whose form is regulated by growth stimulating and inhibiting substances as demonstrated by BURNETT (1966) and substantiated by MACKLIN (1968). Considering the antagonism between fission and regeneration as well as the infectious spreading of transverse fission, it seems that similar form-regulating substances may be operative in *A. stellula.* One substance produced by dividing specimens may stimulate other individuals to undergo fission; another substance produced by regenerating anemones could render them incapable of division whilst regenerating. The aquarium conditions were such that both materials could accumulate, thus increasing the rate of transverse fission among non-dividing anemones and permitting them to regenerate distal structures. BRODERICK (1860) has likewise observed fission within a period of a few days in all 3 anemones maintained in his aquarium.

The results of experiment 1 also suggest that small amounts of materials which stimulate fission and regeneration are present in non-dividing *Anthopleura stellula*, and that small transverse incisions made below the pharynx enhance the activity of these substances, resulting in immediate fission and regeneration. MOSZKOWSKI (1907) and CHILD (1909) consistently obtained wound healing as normal reaction to incising anemones below the pharynx; this reaction has been observed in *A. stellula* only after raising the salinity.

The species' specifity of these inducer materials may explain the indifference of the 2 asexually reproducing species to their presence (experiment 4). However, there were some typical reactions in *Bunodactis verrucosa*: the slit-like opening, the regeneration of tentacles, and the dedifferentiation which proceeded horizontally. The warts of this species disappeared like those in the proximal region of the regenerating distal part of *Anthopleura stellula.* It is possible that the regulatory substances present in the medium containing dividing and regenerating *A. stellula* do not have the same effect upon *B. verrucosa* because this latter species lacks a "budding region". This result clearly demonstrates that any stimulatory materials which exist in the culture medium are rather specific for *Anthopleura stellula.*

Considering the extended period of regeneration in *Bunodactis verrucosa* and the results of experiments 2 and 3, the stimulatory materials probably exert a lasting effect upon anemones. However, individuals which were placed in cold seawater for 2 months failed to undergo fission and regeneration unless they were transferred to warmer water. The cold treatment must have inhibited the long term activity of the regulatory substances, since these anemones could not initiate more than one fission in the warmer water (experiment 3). One would normally expect warm seawater to facilitate further division, since higher temperatures have fostered an increased rate of longitudinal fission in *Anemonia sulcata* (LOUIS, 1960) and *Diadumene luciae* (MIYAWAKI, 1952).

The influence of salinity on fission and regeneration is clearly demonstrated in experiment 5. These results are supported by the experiments of several authors. COTRONEI (1924) has shown that oral disc regeneration in transversely cut cerianthids is completed more quickly in diluted seawater than in higher salinities. MIYAWAKI (1951) likewise obtained the highest rate of longitudinal fission in *Diadumene luciae* when the salinity was slightly lowered. However, LOUIS (1960) found no salinity influence on the longitudinal fission of *Anemonia sulcata.*

Abrupt increases in salinity seem to either destroy the fission-inducing substances, or to interfere with their stimulatory effects on transverse fission; thus, the fission process is interrupted even in dividing anemones. Regeneration only takes place in those portions of the proximal part which have separated from the distal column (Fig. 2 f, g). However, it should be noted that the Mediterranean species *Bunodactis verrucosa* reacted to the medium containing dividing and regenerating specimens of *Anthopleura stellula* kept at normal levels of Mediterranean salinity. This indicates that the stimulatory substances have a direct influence upon sea anemones not exposed to a lower salinity. Decreases in salinity, therefore, only have an indirect supportive effect on the fission process.

Pedal disc regeneration in sea anemones has been the object of much discussion. MOSZKOWSKI (1907) thought that an inhibitory factor controls its formation, while CARLGREN (1909, 1926, 1929) and ABELOOS (1955) maintained that the anemone's distal part completely lacks the capacity to regenerate a pedal disc. On the other hand, HAZEN (1903), LANDAUER (1925, 1926) and KOTODZIEJSKI (1932) observed that pedal discs could form in the distal region following artificial amputation.

All distal parts regenerated a pedal disc, without exception, in the medium containing dividing anemones, while this capacity was lost after exposure to low temperature or higher salinity. There seems to be no inhibitory factor, but rather a deficiency of a substance which stimulates pedal disc regenerationin this case.

There are several different views about pharyngeal regeneration in amputated sea anemones. MOSZKOWSKI (1907) and CARLGREN (1929) found that the pharynx is restored by invaginating ectoderm. Similarly, CHILD (1903a) stated that pharyngeal formation in *Cerianthus solitarius* proceeds from the directive radius of the oral disc (ectoderm). However, ABELOOS (1958) found that endodermal pharyngeal restitution occurs without participation of the filaments and independent of the "symétrisation" of the mesenteries. Using amputated pieces of *Diadumene luciae* "in which a mesentery was left in one particular region" HAZEN (1902) observed pharyngeal formation by the endodermal layers near the oral end. Presumably, this mesentery was a directive, and HAZEN observed the same events in pharyngeal formation as described in this report. The regeneration of the pharynx is induced by the directives because the farthest proximal extension of the pharynx and the directive filaments occurs at this mesentery pair (compare p. 251). Apart from this, pharyngeal formation extends horizontally only as far as the area of growing mesenteries and budding tentacles (Fig. 3d). It is rather difficult, if not impossible, to decide whether or not filaments are transformed into pharyngeal tissue during this process. In normal non-regenerating anemones a gradual transition occurs between both types of tissues (STEPHENSON, 1928). These relationships are supported by my observations, which demonstrate the occurrence of the p-rhabdoides A in only the filaments and the pharynx of *Anthopleura stellula* and several other actinians (SCHMIDT, 1969).

There is also some question about the possibility that *Anthopleura stellula* habitually reproduces by transverse fission in nature. Since Mediterranean levels of salinity have never been recorded in the Gulf of Acquaba, one would not expect that transverse fission in this anemone occurs naturally as a result of low levels of salinity. However, it has been shown that transverse fission is species specific and may occur in normal salt concentrations: it should be noted that the inducing substances, once produced, may be operative in sea anemones not treated with reduced salinities (as demonstrated in this paper on specimens of *Bunodactis verrucosa*). The most important factor in the induction of the fission process seems to be the accumulation of the stimulatory substances in the surrounding water. Without such an accumulation, transverse fission could only occur sporadically in the sea as does longitudinal fission.

Since viviparously or asexually reproducing sea anemones live clumped together (SCHMIDT, 1967), it is possible for sufficient stimulatory substances to accumulate and promote the fission process. Sex and oral disc pattern are not changed by transverse fission. These statements support the hypothesis that transverse fission occurs in *Anthopleura stellula* in nature: males and females collected at Eilat had rather different oral disc patterns; however, in individuals of the same sex they were identical. It should also be mentioned that asexual reproduction via longitudinal fission is quite common within the genus *Anthopleura* (STEPHENSON, 1935; HAND, 1955; DAVIS, 1962).

Summary

1. *Anthopleura stellula* EHRENBERG, 1834, a sea anemone from Eilat, Red Sea, has been redescribed in detail and is found to be synonymous with *Anthopleura elatensis* ENGLAND 1969.

2. Autonomous transverse fission (by architomy) is observed when *A. stellula* is maintained at Mediterranean salinities (37.0 to 37.5$^0/_{00}$) for about 5 months.

3. Fission is always performed at the same level of the body column; it spreads infectiously amongst other sea anemones and is repeated by divided anemone parts after they have regenerated.

4. Maintenance of the Mediterranean species *Bunodactis verrucosa*, a sea anemone which does not reproduce asexually, in the medium containing dividing *Anthopleura stellula*, induces half a transverse fission and regeneration, followed by dedifferentiation.

5. When non-dividing *Anthopleura stellula* are transferred from an aquarium containing specimens actively engaging in transverse fission to fresh Mediterranean water, they begin to undergo fission after 11 days.

6. Full pedal disc regeneration has been obtained in an aquarium containing dividing and regenerating specimens. Pharynx formation is induced by the directive mesenteries of the first budding tentacle. The oral disc pattern is restored in detail by the proximal part.

7. When specimens, about to divide, are exposed to low temperatures (12° to 13 °C) for 2 months, their capacity for pedal disc regeneration and repeated fission is lost.

8. Abrupt increase in salinity from 37.0 to 37.5$^0/_{00}$ to the level of the Red Sea (40.0 to 41.0$^0/_{00}$) interrupts fission of dividing anemones and also prevents pedal disc regeneration.

9. Anemones incised transversely at the usual level of fission promptly perform transverse fission and regeneration in the medium containing dividing specimens. After increasing the salinity, such treatment results in wound healing rather than fission, and regeneration (of the proximal part) in 9 out of 10 cases.

Acknowledgements. I am indebted to MAMBO, especially to Dr. A. DOHRN (Naples, Italy). Dr. R. ASHBEL and Dr. F. D. POR (Jerusalem, Israel), as well as to the staff of the Zoological Institute of the Hebrew University (Jerusalem, Israel) who arranged the course at Eilat which gave rise to this study.

I sincerely thank Professor Dr. Dr. h. c. W. E. ANKEL (Giessen, FRG) for critically reading the manuscript, and Professor Dr. A. BURNETT (Evanston, USA) for helpful discussions. Further, I wish to express my sincere thanks to Dr. I. SINGER

(New York, USA), who has advised me in regard to the English text. My thanks are also extended to Dr. Dzwillo (Hamburg, FRG) and to Dr. Kühlmann (Berlin, GDR) for preparing the type specimens.

This study was performed at the Stazione Zoologica di Napoli, Italy. Financial support was generously provided by "Anton und Reinhard Dohrn-Stiftung" "DAAD" and "Stiftung Volkswagenwerk-Stipendien".

Literature cited

Abeloos, M. M.: Régénération et morphogénèse chez *Actinia equina* L. C.r. Ass. fr. Avanc. Sci. **74**, 361—363 (1955).

— Sur la „dominance“ de la bouche des actinies. C. r. hebd. Séanc. Acad. Sci., Paris **247**, 139—141 (1958).

Andres, A.: Intorno alla scissiparità delle attinie. Pubbl. Staz. zool. Napoli (Mitt. zool. Stn Neapel) **3**, 124—148 (1882).

— Le attinie. Fauna Flora Golf. Neapel **9**, 1—459 (1884).

Blochmann, F. and C. Hilger: Über *Gonactinia prolifera* Sars, eine durch Quertheilung sich vermehrende Actinie. Morph. Jb. **13**, 385—401 (1888).

Brandt, J. F.: Prodromus descriptionem animalium ab H. Mertensio in orbis terrarum circumnavigatione observatorum. Recl Act. Séance sol. Acad. imp. Sci. St. Pétersbourg 201—275 (1835).

Broderick, F. N.: On the transverse fission of *Aiptasia couchii*. Zoologist **18**, 6911—6912 (1860).

Burnett, A. L.: A model of growth and cell differentiation in *Hydra*. Am. Nat. **100**, 165—190 (1966).

Carlgren, O.: Ostafrikanische Aktinien. Mitt. naturh. Mus. Hamb. **17**, 21—144 (1900).

— Studien über Regenerations- und Regulationserscheinungen. II. Ergänzende Untersuchungen an Actiniarien. K. svenska Vetensk-Akad. Handl. **43**, 3—48 (1909).

— Zur Regeneration der Actiniarien. Z. Morph. Ökol. Tiere **5**, 150—154 (1926).

— Zur Biologie und Regeneration der niederen Actiniarien. Z. Morph. Ökol. Tiere **14**, 522—530 (1929).

— A survey of the *Ptychodactiaria*, *Corallimorpharia* and *Actiniaria* K. svenska Vetensk-Akad. Handl. **1**, 1—121 (1949).

Child, C. M.: From regulation in *Cerianthus*. 1. The typical course of regeneration. Biol. Bull. mar. biol. Lab., Woods Hole **5**, 239—260 (1903a).

— From regulation in *Cerianthus*. 2. The effect of position, size and other factors upon regeneration. Biol. Bull. mar. biol. Lab., Woods Hole **5**, 304—319 (1903b).

— Factors of form regulation in *Harenactis attenuata*. 1. Wound reaction and restitution in general and the regional factors in oral restitution. J. exp. Zool. **6**, 471—506 (1909).

Cotronei, G.: Doppie formazioni, scissiparità e rigenerazioni nelle attinie. Pubbl. Staz. zool. Napoli **5**, 199—273 (1924).

Davis, C.: One equals two. A sea anemone achieves its plurality in singular fashion. Nat. Hist., N.Y. **71**, 61—63 (1962).

Ehrenberg, C. G.: Die Corallenthiere des rothen Meeres. Berlin: Königl. Akad. Wiss. 1834.

England, K. W.: *Anthopleura elatensis* n. sp. (Actiniidae: Actiniaria) from the Red Sea. Israel J. Zool. **18**, 1—7 (1969).

Hand, C.: The sea anemones of central California. Part 2. The endomyarian and mesomyarian anemones. Wasmann J. Biol. **13**, 37—99 (1955).

Hazen, A. P.: The regeneration of an oesophagus in the anemone, *Sagartia luciae*. Arch. EntwMech. Org. **14**, 592—599 (1902).

— Regeneration in the anemone, *Sagartia luciae*. Arch. EntwMech. Org. **16**, 365—376 (1903).

Holdworth, E. W. H.: On the transverse fission of *Aiptasia couchii*. Zoologist **18**, 6945—6946 (1860).

Kerb, H.: Studien über die ungeschlechtliche Fortpflanzung der *Gonactinia prolifera* Sars. Bergens Mus. Årb. **3**, 1—14 (1913).

Klunzinger, C. B.: Die Korallthiere des rothen Meeres. Berlin: Gutmann 1877.

Kotodziejski, M. Z.: Untersuchungen über die Regeneration der Fußscheibe bei *Actinia equina* L. Bull. int. Acad. pol. Sci. Lett. (Ser. B) 153—187 (1932).

Landauer, W.: Laceration, Knospung und Heteromorphose bei *Actinia equina* L. Z. Morph. Ökol. Tiere **3**, 177—187 (1925).

— Zur Regeneration der Actiniarien. Z. Morph. Ökol. Tiere **6**, 586—587 (1926).

Laubier, L. and H. Schmidt: Le genre *Staurosoma* Will (copépode parasite d' actinies) en Mer Rouge Crustaceana (In press).

Louis, C.: Modalités et déterminisme expérimental de la scissiparité chez l'actinie *Anemonia sulcata* Pennant. C. r. hebd. Séan. Acad. Sci., Paris **251**, 134—136 (1960).

Macklin, M.: Analysis of growth factor gradients in *Hydra*. J. cell. comp. Physiol. **72**, 1—8 (1968).

Milne Edwards, H.: Histoire naturelle des coralliaires ou polypes proprement dits. Vol. 1. 326 pp. Libraire encyclopédique de Roret, Paris 1857.

Miyawaki, M.: Notes on the effect of low salinity on an actinian *Diadumene luciae*. J. Fac. Sci. Hokkaido Univ. (Ser. 6) **10**, 123—126 (1951).

— Temperature as a factor influencing upon the fission of the orange-striped sea-anemone, *Diadumene luciae*. J. Fac. Sci. Hokkaido Univ. (Ser. 6) **11**, 77—80 (1952).

Moszkowski, M.: Die Ersatzreaktionen bei Aktinien (*Actinia equina* und *Actinoloba dianthus*). Arch. EntwMech. Org. **24**, 411—433 (1907).

Oren, O. H.: A note on the hydrography of the Gulf of Eylath. Bull. Sea Fish. Res. Stn **30**, 3—14 (1962).

Prouho, H.: Observations sur la *Gonactinia prolifera* (Sars) draguée dans la Méditerranée. Archs Zool. exp. gén. (Ser. 2) **9**, 247—254 (1891).

Safriel, U. and Y. Lipkin: Note on the intertidal zonation of the rocky shores at Eilat (Red Sea, Israel). Israel J. Zool. **13**, 187—190 (1964).

Sars, M.: Beskrivelser og iagttagelser over nogle merkelige eller nye i havet ved den Bergenske kyst levende dyr af Polypernes, Acalephernes, Radiaternes, Annilidernes og Molluskernes classer. Bergen: 1835.

Schmidt, H.: A note on the sea-anemone *Bunodactis verrucosa* Pennant. Pubbl. Staz. zool. Napoli **35**, 252—253 (1967).

— Die Nesselkapseln der Aktinien und ihre differentialdiagnostische Bedeutung. Helgoländer wiss. Meeresunters. **19**, 284—317 (1969).

Singer, I. I. and J. D. Palmer: Tentacular and oral-disc regeneration in the sea anemone, *Aiptasia diaphana*. 1. Sequential morphological events in distal-end restitution. J. Morph. **127**, 373—382 (1969).

Stephenson, T. A.: The British sea anemones. Pt 1. London: The Ray Soc. 1928. (Monogr. Ray Soc. 113).

— The British sea anemones. Pt 2. London: The Ray Soc. 1935. (Monogr. Ray Soc. 121).

Will, F.: Über *Staurosoma*, einen in den Aktinien lebenden Schmarotzer. Arch. Naturgesch. **10**, 337—342 (1844).

Author's address: Dr. H. Schmidt
1. Zoologisches Institut
der Justus Liebig-Universität
Stephanstraße 24
63 Giessen, Germany (FRG)

Date of final manuscript acceptance: February 6, 1970. Communicated by O. Kinne, Hamburg