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Original Article

Control of formation of the two types of polyps in *Thecocodium quadratum* (Hydrozoa, Cnidaria)

RENATE PFEIFER and STEFAN BERKING*

Zoologisches Institut, Universität zu Köln, Köln, Germany

ABSTRACT ***Thecocodium quadratum* (Werner, *Jber. Biol. Anst. Helgoland,* 1965) is a colonial hydroid which produces 2 different types of polyps: gastrogonozooids and dactylozooids. The mouthless dactylozooids bear tentacles and catch the prey, which is then taken over and swallowed by the gastrogonozooids which have no tentacles. It is obvious that for a colony to survive both polyps must exist simultaneously arranged in a certain spatial pattern. Our experiments indicate that the formation of polyps in a growing culture is governed by at least 3 principles: (1) short range inhibition between polyps irrespective of their differentiation; (2) long range specific inhibition between gastrogonozooids; and (3) long range supporting influence (lateral help, Meinhardt, H., *Models of Biological Pattern Formation,* 1982) between gastrogonozooids and dactylozooids.**

KEY WORDS: *Thecocodium, hydrozoa, polymorphism, pattern formation, sinefungin*

Introduction

The colonies of most hydroids consist of large numbers of polyps connected by hollow tubes termed stolons. Such colony grows by elongation and lateral branching of the stolons. On top of the new stolons polyps develop at more or less regular intervals. The control of spacing is proposed to include diffusible inhibitors generated by polyps (Bravermann, 1971; Müller, 1984; Berking, 1986; Plickert *et al.*, 1987). Usually colonies of hydroids contain simultaneously different types of polyps. In most cases only one type is able to feed while the others have different functions, e.g. sexual reproduction. The food is taken up by the polyps termed gastrozooids or trophozooids and is transported in a partially digested form through the stolons to all parts of the colony including to those polyps which cannot feed themselves. Generally, young colonies contain only those polyps which can feed, the other types are formed later in the ontogeny. Often the latter types are formed only in some parts of the colony.

The polymorphism of *Thecocodium quadratum* is different, it is obligatory (Werner, 1965; Bouillon, 1967; Jarms, 1987). Dactylozooids have tentacles but no mouth. They can catch prey but are not able to feed. Gastrogonozooids have no tentacles but have a mouth. They take up the prey that is caught by the dactylozooids. Thus it is easy to see that both types must be present simultaneously in a certain spatial pattern. The gastrogonozooids of a colony produce either male or female medusae. The medusae produce the gem cells. Fertilized eggs develop into planula larvae. Unfortunately up to now sexually mature medusae have not yet been grown up in the laboratory. Therefore, the architecture of the founder of a colony, the primary polyp which develops by metamorphosis from a larva, is unknown.

The aim of our study is to get insight into the mechanism which determines the formation and the final spatial pattern of 2 types of polyps which functionally depend on each other.

Results

Polyp dimorphism in Thecocodium quadratum

The colony of *Thecocodium* consists of a net of hollow tubes termed stolons (diameter about 50 μm) on top of which 2 types of polyps – dactylozooids and gastrogonozooids – are formed. The dactylozooids are slim and small (about 150 μm long). They bear in almost every case 4 tentacles but no mouth (Fig. 1A). Dactylozooids catch the prey, in our laboratory nauplii of *Artemia salina* which are 5 times larger then the polyps, but cannot swallow them. Gastrogonozooids (Fig. 1A) are much larger (2000-3000 μm long) than dactylozooids and have a mouth but no tentacles (in 2 cases one tentacle of the type formed by dactylozooids was present transiently). When a prey is killed and fixed by the short tentacles of a dactylozooid the gastrogonozooids in its vicinity start to stretch into the direction of the fixed prey, probably guided by agents released by the prey or the dactylozooid into the sea water. When a gastrogonozooid's mouth touches the fixed prey it opens and starts to swallow the prey. The feeding range of a gastrogonozooid is 2000-3000 μm.

It appears that dactylozooids are not simply developmentally-retarded gastrogonozooids. We found the nerve net to be quite differently organized in the polyps by staining the RFamide-positive nerve cells. Dactylozooids bear RFamide-positive pericarya in the terminal thickening of a tentacle, and neurites along the body column (Fig. 1B). In gastrogonozooids the RFamide-positive neurites run mostly parallel to the length axis of the body. Pericarya

***Address for reprints:** Zoologisches Institut, Universität zu Köln, Weyertal 119, 50923 Köln, Germany. FAX: 221.4705171.

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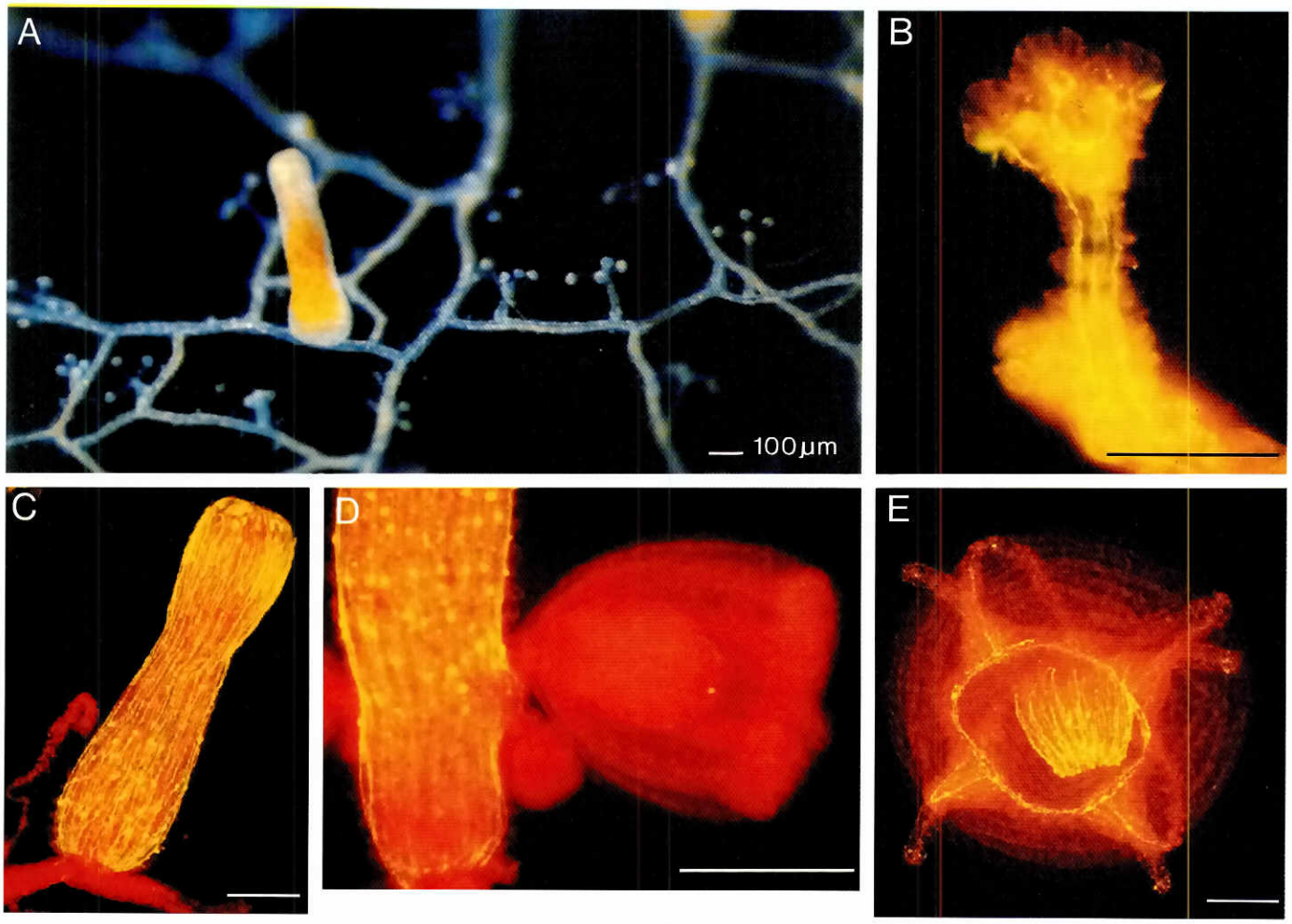

Fig. 1. *Thecocodium quadratum*. (A) *View on a colony consisting of 2 types of polyps connected by hollow tubes termed stolons. There can be seen several dactylozooids with 4 tentacles and one large gastrogonozooid without tentacles. RFamide-positive nerve cells are shown in* **(B)** *a dactylozooid and* **(C)** *a gastrogonozooid. Note that the stolon is free of these nerve cells.* **(D)** *A gastrogonozooid producing a medusa. The medusa is free of these nerve cells, with exception of the tip of its manubrium where one perikaryon has just become visible.* **(E)** *RFamide-positive nerve cells in a young medusa. The bars in all figures represent 100 mm. Fig. 1A was made by H.J. Hoffmann.*

are found close the mouth opening and in the basal half of the body, often arranged within 3 rings (Fig. 1C). Gastrogonozooids in the central part of a colony produce medusae. Even though a medusa is formed from gastric tissue which contains RFamide-positive nerve cells it does not contain these cells or neurites at early stages, but after separation from the gastrogonozooid it does (Fig. 1D,E). Stolons are free of RFamide-positive cells (Fig. 1B).

In a colony the dactylozooids are much more frequent than gastrogonozooids. We measured a ratio of 12:1, 14:1 and 9:1 in experimental colonies bearing 53, 61 and 71 polyps, respectively. The mean minimal distance between 2 dactylozooids or between a dactylozooid and a gastrogonozooid was about 400 µm, whereas the distance between 2 gastrogonozooids was 4 to 5 times greater (cf. Fig. 1A).

Colonies grow by elongation of the stolons and by lateral stolon branching (Figs. 1A, 2). In the periphery, either a gastrogonozooid or a dactylozooid forms on top of the stolon some 100 µm away from the stolon tip. We have the impression that when a gastrogonozooid forms in the colony's periphery some distance away from the other polyps, it quickly becomes surrounded by some dactylozooids, while when a dactylozooid forms in the very periphery, neither dactylozooid nor gastrogonozooid formation is stimulated close to it (Fig. 2).

To get a deeper insight into the interaction between dactylozooids and gastrogonozooids we produced various forms of isolates and studied the conditions and kinetics of the formation of new polyps and the regression of existing polyps.

Conditions for stability and regression of dactylozooids

Within a large colony dactylozooids did not regress for at least one week of starvation (53 out of 53). However, isolated polyps regressed rapidly when separated from the colony by a cut through the stolons some distance away from the base of the polyp: 85% regressed within 3 days (Table 1). During regression the stolons at the base of the polyp elongated indicating that polyp tissue was transformed into stolon tissue.

Isolates containing 3 dactylozooids regressed faster that those containing one dactylozooid (Table 1). When, however, the 3 dactylozooids were connected with a gastrogonozooid, regression did not take place (Table 1). Dactylozooids with a large piece of

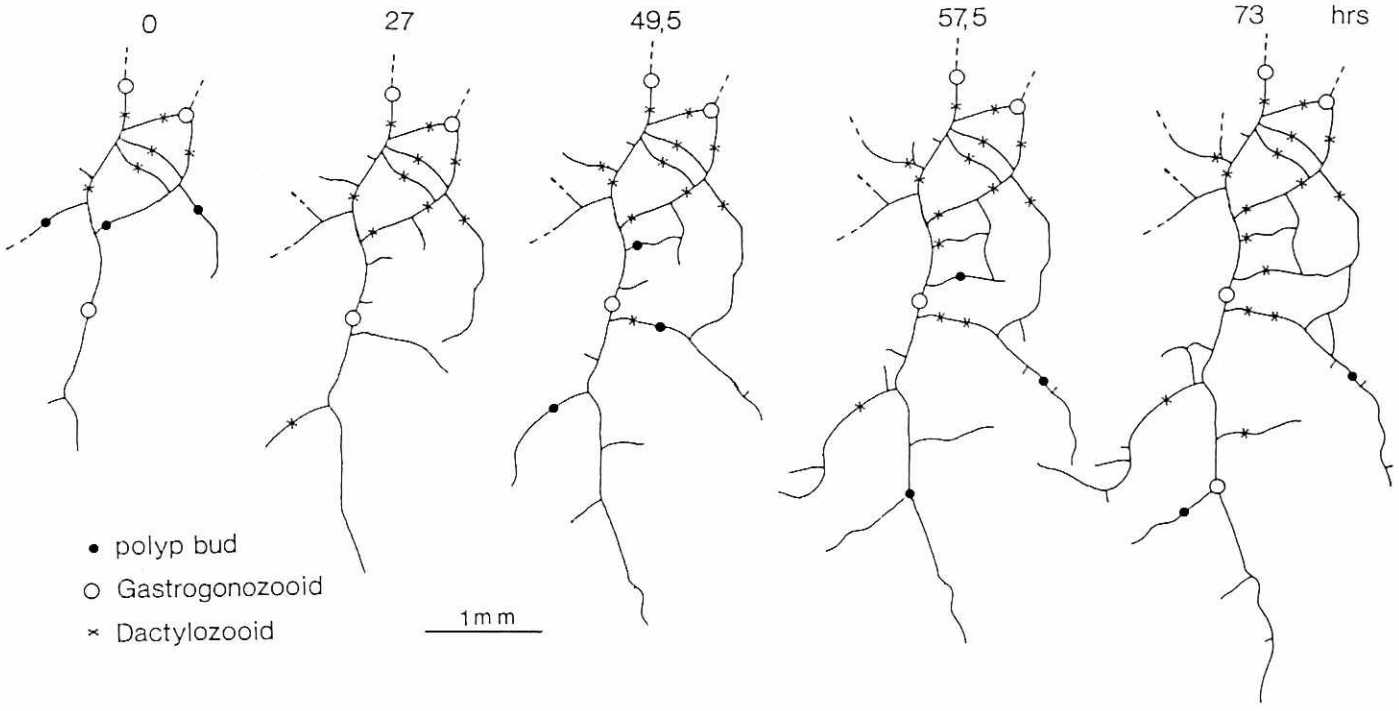

Fig. 2. Growth at the periphery of a colony of *Thecocodium quadratum*.

stolon at their base regressed faster than dactylozooid with a small piece of stolon (Table 1).

Conditions for stability and regression of gastrogonozooids

Isolated gastrogonozooids regressed while a gastrogonozooid within a colony is stable for at least 8 days. However, the process of regression took several days (Table 1), possibly because a gastrogonozooid is much bigger than a dactylozooid. A row of 5 dactylozooids was found to have a significant stabilizing influence on a gastrogonozooid (Table 1).

The decision of a gastrogonozooid to regress appears to occur early after isolation; gastrogonozooids were isolated from the colony by removing connecting stolon tissue. When the gap was small (about 600 µm) the isolates reunited with the colony within one day (29 out of 30). However, in several cases even this rapid reunion did not prevent regression, which occurs several days later (Table 2). It appears that for a gastrogonozooid to survive a continuous contact to the colony is necessary.

Conditions of gastrogonozooid and dactylozooid formation

If all gastrogonozooids of a colony (14 experimental colonies) are removed new gastrogonozooids are formed within 2 to 3 days. We removed not only the polyps but also a small part of the adjacent stolon tissue to make sure that the new gastrogonozooids did not form from remaining gastrogonozooid tissue but rather from stolon tissue. Dactylozooids were not formed, but rather some degenerated during that time. In untreated colonies the formation of gastrogonozooids was not observed during this time (not shown).

The type of polyp which forms first in an isolate depended strongly on the type of polyps already present in the isolate. When there were only dactylozooids present a gastrogonozooid formed first with high frequency. When there was a gastrogonozooid present even in addition to 10 dactylozooids a further dactylozooid formed in all cases (Table 3).

Feeding was found to have strong influence on polyp formation. In all experiments reported above the animals were not fed during the experiment. We found that the first polyp which was formed on the elongating stolons of an isolated starved gastrogonozooid was

TABLE 1

STABILITY OF ISOLATED DACTYLOZOOIDS (DZ) AND GASTROGONOZOOIDS (GZ)

Type and number of polyps in the isolates	Frequencies (%) of isolates in which at least one DZ or GZ regressed up to day						Number of isolates
	1 DZ	2 DZ	3 DZ	6 GZ	7 GZ	8 GZ	
1 DZ	23	62	85	-	-	-	13
1 DZ control *	0	0	3	-	-	-	10
1 DZ	9	-	-	-	-	-	76
3 DZ	67	-	-	-	-	-	24
3 DZ	51	-	-	-	-	-	43
3 DZ + 1 GZ	0	-	-	-	-	-	45
1 DZ (100 µm) **	0	35	-	-	-	-	28
1 DZ (700 µm)**	52	78	-	-	-	-	23
1 GZ	-	-	-	27	33	82	30
1 GZ control*	-	-	-	0	0	3	30
1 GZ	-	-	-	-	-	33	51
1 GZ + 5 DZ	-	-	-	-	-	13	39

-, not determined; *, control, refers to polyps within the colony; **, length of stolon at the base of the polyp. Frequencies of pairs are significantly different (Fisher-Yates-Test, 5% level).

TABLE 2

STABILITY OF INITIALLY ISOLATED GASTROGONOZOOIDS (GZ) FOLLOWING REUNION WITH THE COLONY

Type of isolate	Frequencies (%) of isolates with no GZ at		Number of isolates
	day 6	day 10	
GZ within the colony, not isolated	0	10	30
Isolates which did not reunite with the colony	27	82	30
Isolates which reunited with the colony within one day, initial gap size to colony about 600 µm	37	10*	30
Isolates which reunited with the colony in less than 1 day, initial gap size about 100 µm	3	!7*	30

*10 days after isolation some initially isolated pieces have developed a new gastrogonozooid close to the position of the old regressed one. At day 6 the frequencies of group 2 and 3 differ significantly (Fisher-Yates-Test, 5% level) from those of groups 1 and 4.

in all cases a dactylozooid. However, when the gastrogonozooids were fed, 20% of the isolates produced at first a further gastrogonozooid (Table 4). The polyps were fed with freshly minced *Artemia* because they were not able the catch living *Artemia*. In isolates containing one gastrogonozooid feeding antagonized dactylozooid formation (Table 5).

In the hydrozoa *Eirene viridula* polyp formation takes place at a longer distance when the (endogenous) methyl donor homarine was applied externally and at shorter distance when sinefungin – an antagonist of transmethylation – was applied. Therefore, it was suggested that spacing is controlled by endogenous methyldonors making use of S-adenosylmethionine, the most important methyldonor of animal tissue (Berking, 1986). We treated colonies consisting of one gastrogonozooid and 3 dactylozooids with sea water containing 1 µM Sinefungin for 3 days and found that 80% of the treated isolates but only 20% of the untreated ones produced a dactylozooid (Table 6).

Discussion

In hydrozoa the stolon tissue is among the most potent tissues. It produces stolon buds laterally and polyp buds on top. In both cases a certain spacing takes place. The mechanism of polyp spacing is proposed to include diffusible substances which are able to prevent polyp formation. The substances are proposed to be generated by polyps (Braverman, 1971; Müller, 1984; Berking, 1986; Plickert *et al.*, 1987). This assumption explains why polyp formation is possible only at a certain distance from an existing polyp. Gierer and Meinhardt (1972) showed that spacing of structures can also result when the structure depletes the surrounding tissue of a substance which is necessary for its formation and maintenance. When in the case of colonial hydroids such substance is produced everywhere, is diffusible, is necessary for polyp formation, and is used up by polyps, the formation of further polyps in the close vicinity of existing ones is prevented, while their formation at a greater distance is possible.

In the hydroids *Hydractinia* and *Eirene* endogenous methyldonors, including homarine, trigonelline and betaine, were proposed to be used as polyp inhibitors. Homarine applied exogenously in a low concentration increased the distance between a polyp and the next polyp bud on the same stolon. On the other hand, the antibiotic sinefungin, which is an inhibitor of transmethylation, allows polyp formation at a shorter distance to an existing polyp. On the basis of this observations it was proposed that the methyldonors act via production of S-adenosyl methionine (SAM), the most important methyldonor in organisms. The noted methyldonors may be secreted by a polyp into the stolons (Berking, 1986).

TABLE 3

FORMATION OF GASTROGONOZOOIDS (GZ) AND DACTYLOZOOIDS (DZ) IN LARGE ISOLATES

Isolates containing	Frequencies (%) of isolates forming first a GZ	DZ	GZ or DZ	Number of isolates
10 GZ	31	3	66	32
10 DZ + 1 GZ	0	100	0	34

The term "GZ or DZ" refers to isolates which formed both types of polyps between the daily intervals of observation. The frequencies in the groups are significantly different (Fisher-Yates-Test, 5% level).

TABLE 4

INFLUENCE OF FEEDING ON POLYP FORMATION ON STOLONS THAT GROW OUT FROM ISOLATED GASTROGONOZOOIDS (GZ)

Type of treatment	Frequencies (%) of isolates forming first a GZ	DZ	GZ or DZ	Number of isolates
starved	0	100	0	32
fed	20	52	28	66

The term "GZ or DZ" refers to isolates which formed both types of polyps between the intervals of observation .The frequencies in the groups are significantly different (Fisher-Yates-Test, 5% level).

In *Thecocodium quadratum* 2 types of polyps are formed, dactylozooids and gastrogonozooids. Thus, there must exist not only a mechanism which controls spacing of polyps, but also one deciding which type of polyp is formed. The mean minimal distance between 2 dactylozooids equals that between a dactylozooid and a gastrogonozooid, while the mean minimal distance between 2 gastrogonozooids is much greater. We argue that there exists (1) a short range inhibition between polyps irrespective of their differentiation. The finding that sinefungin enhances dactylozooid formation may indicate that also in *Thecocodium quadratum* methyldonors play a key role in the spacing of polyps. We argue that the methyldonors are involved in this short range inhibition. (2) In addition to this short range inhibition, there appears to exist a long range specific inhibition between gastrogonozooids.

The assumption of these 2 control mechanisms is not sufficient to explain the data obtained. We found that in isolates the formation and maintenance of a polyp depends on the existence of a polyp

TABLE 5

INFLUENCE OF FEEDING ON DACTYLOZOOID (DZ) FORMATION IN ISOLATES CONTAINING ONE GASTROGONOZOOID (GZ)

Type of treatment	Frequencies (%) of isolated GZ which produced at least one DZ within 7 days after isolation	Number of isolates
starved	49	47
fed	13	55

The frequencies in the groups are significantly different (Fisher-Yates-Test, 5% level).

of the other type. Dactylozooid formation is stimulated when a gastrogonozooid is present, while gastrogonozooid formation is stimulated when only dactylozooids are present. An isolated dactylozooid will survive only if it is in contact via a stolon with a gastrogonozooid. It appears that some sort of lateral help (Meinhardt, 1982) exists between polyps of different specificity. Because of the long range of inhibition between 2 gastrogonozooids the experiments are conclusive only for unilateral support: gastrogonozooids may at least help dactylozooids to form and to survive. There is one observation which fits better into this type of unilateral help than into mutual help. When a gastrogonozooid is formed in a colony's periphery the formation of dactylozooids appears to be stimulated in its vicinity, but when a dactylozooid is formed some distance away from the other polyps it appears not to enhance the formation of gastrogonozooids (and dactylozooids) in its vicinity.

There are two further observations which may help to understand pattern control. Stolons showed an unexpected influence on the stability of dactylozooids. The frequency and speed of regression of a dactylozooid was higher the larger the piece of stolon at the base of the isolated polyp. Feeding of an isolated gastrogonozooid results in its stabilization and strongly stimulates the formation of a further gastrogonozooid. At the same time feeding inhibits dactylozooid formation in isolates containing a gastrogonozooid and a dactylozooid. Therefore feeding stimulates the formation of those polyps which can feed and inhibits the formation of those which only can catch the prey.

Based on these data at least two alternative models of specific inhibition and lateral help can formulated which imply quite different strategies for a biochemical approach to the problem. (1) In addition to an unspecific lateral inhibition of polyps by polyps, probably including secreted methyldonors (see above), stolon tissue is proposed to produce a diffusible agent necessary for gastrogonozooid formation and stability. The observed specific inhibition of gastrogonozooid formation close to an existing one is due to the uptake of the agent by the polyp from the stolons causing its depletion there. This very agent antagonizes somehow dactylozooid formation. Based on these assumptions, stolons in the colony's periphery are expected to contain the hypothetical agent in a mean concentration which allows a scattered production of both dactylozooids and gastrogonozooids, as it is observed. When in the colony's periphery a dactylozooid is formed, we found no tendency for a further dactylozooid or gastrogonozooid to form close to it, but when a gastrogonozooid is formed dactylozooids develop close to it. According to the model, this happens due to the reduced availability of the hypothetical agent. Feeding may contribute to the supply of the colony with the hypothetical agent. Dactylozooids may produce the hypothetical agent as well. (2) In the alternative model it is also proposed that polyps inhibit polyp formation unspecifically in their close vicinity. Further, gastrogonozooids are proposed to produce a substance (with long range) which interferes with the formation of gastrogonozooids. This substance is required by dactylozooids. In its absence, dactylozooids degenerate and new ones are not formed. Feeding may cause a loss of the hypothetical agent from stolon tissue or a reduced secretion from gastrogonozooid tissue into the stolons. Stolons may produce the agent at a low rate.

TABLE 6

INFLUENCE OF SINEFUNGIN ON DACTYLOZOOID (DZ) FORMATION ON ISOLATES CONTAINING ONE GASTROGONOZOOID (GZ) AND THREE DACTYLOZOOIDS (DZ)

Type of treatment	Frequencies (%) of isolates forming at least one DZ within 3 days after isolation	Number of isolates
sea water	20	29
1 µm sinefungin in sea water	80	30

The frequencies in the groups are significantly different (Fisher-Yates-Test, 5% level).

Materials and Methods

The experimental animals were a gift of G. Jarms (Zoologisches Institut, Hamburg, Germany). Since they were found in the north-east of Mombasa, Kenya (Werner, 1965), they were reproduced asexually in the laboratory. *Thecocodium quadratum* was cultivated in Petri dishes in artificial sea water (Tropic Marine Neu, Tagis Aquarium, Dreieich, Germany) at 23°C in darkness, pH 8,2; 1000 mosmol. They were fed every 2-3 days with at least 2 day-old nauplii of *Artemia salina*. The animals used in our experiments were explants from one colony. On the day before an experiment they were fed for the last time. During experiments the animals were not fed, if not stated otherwise.

Analysis of colony growth

Parts of the colonies were drawn on successive days by means of a drawing tube mounted on a stereomicroscope.

Isolation experiments

Single polyps or groups of polyps were isolated from the colony by cutting the stolons and by scraping away stolon material. leading to a gap between the isolate and the main colony. Several groups of isolates were made within the same dish representing various experimental conditions. At least triplicates were produced for one experiment.

Labeling of RF-amide-positive nerve cells

Cells that contain peptides with Arg-Phe-amide at the C-terminus were detected in whole-mount preparations with an antiserum (Grimmelikhuijzen, 1985), a kind gift of C. Grimmelikhuijzen. We used a fluorescein-isothiocyanat (FITC) conjugated anti-rabbit antibody as secondary antibody (dianova, Hamburg, Germany). Counterstaining was performed with Evans Blue.

Statistical analysis

Significance was tested by Fisher-Yates test, 5% level.

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