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ORIGINAL ARTICLE

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On natural metamorphosis inducers of the cnidarians *Hydractinia echinata* (Hydrozoa) and *Aurelia aurita* (Scyphozoa)

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Abstract *Hydractinia echinata* and *Aurelia aurita* produce motile larvae which undergo metamorphosis to sessile polyps when induced by external cues. The polyps are found at restricted sites, *A. aurita* predominantly on rocks close to the shore, *H. echinata* on shells inhabited by hermit crabs. It has been argued that the differential distribution of the polyps in their natural environment largely reflects the distribution of the natural metamorphosis-inducing cues. In the case of *H. echinata*, bacteria of the genus *Alteromonas* were argued to meet these conditions. We found that almost all substrates collected in the littoral to induce metamorphosis in *H. echinata*, and several bacterial strains isolated from the sea, including the common *E. coli*, induce metamorphosis efficiently. In *A. aurita* metamorphosis may be induced by the water–air interface, whereby metamorphosis precedes (final) settlement.

Key words Cnidaria · *Hydractinia echinata* · *Aurelia aurita* · Metamorphosis · Settlement

Introduction

Most cnidarians produce motile larvae which transform in a process called metamorphosis into sessile polyps. The larvae are produced in sexual reproduction either by polyps or by freely swimming medusae (jelly fish). Generally, the polyps are found in restricted areas. With respect to *H. echinata*, which does not produce medusae, the polyp colony is almost exclusively found on shells inhabited by a hermit crab (Schijfsma 1935). With respect to *A. aurita*, the polyps are usually found on rocks close to the shore (Janke 1986) while the medusae are commonly found freely floating in the open sea. In several cnidarians it has been shown that metamorphosis has to be induced by exogenous cues in order to start (Chia and Bickell 1978; Fleck 1997; Leitz 1997; Berking 1998). These cues are delivered by the physiochemical nature of specific surfaces and in particular by so-called biofilms consisting of sedentary bacteria, protozoa, fungi debris, etc. which cover almost all surfaces in marine environments (Fletcher 1994; Holmstroem and Kjelleberg 1994; Herrmann 1996; Hadfield 1998; Wieczorek and Tood 1998). Certain bacteria, present in the biofilm of shells covered by *H. echinata*, have been found to induce metamorphosis (Müller 1969; Spindler and Müller 1972). Wittmann (1977) isolated and identified bacteria of the genus *Alteromonas* as the active principle. Leitz and Wagner (1993) proposed that metamorphosis is induced by certain bacteria occurring, as a rule, on the shells of molluscs inhabited by hermit crabs and isolated *A. espejiana*. We describe here several bacterial strains, including the common *E.coli*, that induce metamorphosis in *H. echinata* efficiently.

In *A. aurita* the aggregated distribution of the polyps is proposed to be a result of the attraction the established polyps have for conspecific larvae (Korn 1966; Groendahl 1989) or the result of a position-dependent recruitment of the larvae within a settling surface (Keen 1987). In addition, our experiments indicate that the water–air interface is able to induce metamorphosis in larvae of *A. aurita*.

For *H. echinata* and for *A. aurita*, the restricted sites at which the polyps are found do not appear to reflect a similarly restricted distribution of specific inducing cues but rather indicate selection after metamorphosis has occurred.

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Materials and methods

Animals

Shells of *Buccinum* spp., with snails still inside, fertile colonies of *H. echinata* on shells of *Buccinum* spp. inhabited by a hermit crab (*Eupagurus* spp.) and fertile medusae of *A. aurita* were ob-

Table 1 Colonisation of *Buccinum* spp. shells

Buccinim spp.	Number of specimens	Shells colonised by *Hydractinia echinata*	Shells colonised by other Hydrozoa, e.g. *Podocoryne carnea*, *Laomedea flexuosa*
Shells inhabited by			
a snail	60	0	60
a hermite crab	70	70	3

Table 2 Distribution of adult *Hydractinia echinata* and distribution of the cues that trigger metamorphosis. One hundred larvae of *H. echinata* competent for metamorphosis were put directly onto the objects to be tested in nonsterile seawater. Experiments were repeated at least three times

Objects	Occurrence of colonies of *H. echinata* under natural conditions	Larvae undergoing metamorphosis when put onto the objects
Buccinum shell inhabited by a snail	–	+*
Buccinum shell inhabited by a hermit crab	+	not tested
Fucus serratus (Phaeophyceae)	–	+
Laminaria spp. (Phaeophyceae)	–	+
Ulva lactuca (Chlorophyceae)	–	+
Stones from littoral	–	+
Petri dish kept in nonsterile seawater for 2 days		+
Petri dish (sterile)		-

+ Indicates >50% metamorphosis; – indicates <5% metamorphosis; +* in the case of *Buccinum* shell inhabited by a snail, all applied larvae which remained on the shell underwent metamorphosis.

tained from the Biologische Anstalt (BA) Helgoland (Helgoland, Germany).

Induction of metamorphosis in *H. echinata* by substrates

The experiments were carried out at the BA Helgoland. Algae or stones for tests of the metamorphosis-inducing capacity were collected in the littoral at the Lange Anna, Helgoland. Fertile colonies of *H. echinata* and shells of *Buccinum* ssp. were collected close by at a depth of about 50 m. About 100 larvae of *H. echinata* competent for metamorphosis were put directly onto the objects to be tested in nonsterile seawater immediately after collection. Collected algae were used as uninjured as possible. As a control, larvae from the same batch were put next to the substrate on the bottom of a (up-to-that-time) sterile dish. Metamorphosis was scored the next day. The experiments were repeated at least three times.

Induction of metamorphosis in *H. echinata* by bacteria

The bacterial strains were isolated from the sea at Helgoland. They were a kind gift of Dr. G. Gerdts (BA Helgoland). The bacteria were grown on agar plates (15 g agar, 5 g yeast extract, 250 ml distilled water and diluted to 1 l with artificial seawater at pH 8) at 18°C for 3–5 days. To test their metamorphosis-inducing capacity, two to three colonies, each of about 10^8 bacteria, were spread with a wooden toothpick on the bottom of a sterile petrie dish (3 cm diameter), washed once with sterile seawater and finally overlaid with 50 larvae in 4 ml sterile filtered seawater. After 3 h, the seawater was changed three times. The yield of metamorphosis was determined the following day. Three repetitions were performed, each in duplicate.

Results

Induction of metamorphosis in *Hydractinia echinata*

In accordance with previous findings (Schijfsma 1935), colonies of *Hydractinia echinata* were found growing on shells inhabited by a hermite crab but not on shells inhabited by a snail (Table 1). However, the metamorphosis-inducing cue was found to be already present on shells inhabited by a snail: when larvae of *H. echinata* were placed on such a shell, they rapidly (within a few hours) underwent metamorphosis (Table 2). Those larvae that happened to fall to the (up-to-that-time) sterile surface of the dish did not undergo metamorphosis. Thus, the material of the shell surface or the biofilm covering this surface is argued to induce metamorphosis.

In order to question the role of the specific surface for metamorphosis induction, including its ability to serve for the suited biofilm, we placed larvae of *H. echinata* on various substrates including stones and algae (*Fucus serratus*, *Laminaria* spp. and *Ulva lactuca*) collected in the littoral of Helgoland (Table 2). In all cases, metamorphosis was induced with high efficiency (>50%, n>100). In sterile dishes metamorphosis did not occur (0%, n>100). Thus, either various substrates are able to cause metamorphosis by themselves or all of them are covered with a biofilm which includes the inducing principle. However, we failed to detect colonies of *H. echinata* on such algae and on stones in the littoral close to the site where colonies were found on the *Buccinum* spp. shells inhabited by a crab.

In a further series of experiments we tested the role of the biofilm for metamorphosis induction by covering an artificial substrate with bacteria. If metamorphosis induction in *H. echinata* can occur almost everywhere, then either the bacteria *A. espejiana* must be present everywhere in sufficient density or other agents including other bacteria also induce metamorphosis. We tested several bacterial strains isolated from the sea and found all of them to induce metamorphosis with high efficiency (Table 3), including gram-negative and gram-positive bacteria and even the common *Escherichia coli*. It appears that the limitation of adult *H. echinata* to hermit

Table 3 Metamorphosis induction in *Hydractinia echinata* by various bacteria. Fifty larvae were treated in 4-ml dishes. Three repetitions were performed, each in duplicate. Highest and lowest yields are shown. Variability of efficiency may be caused by the strongly variable density distribution of bacteria on bottom of dish. Difference between bacteria-treated larvae and those in the two control dishes is significant (χ^2 analysis, 5% level)

Species and objects bacteria	Gram-positive bacteria	Gram-negative	Yield of metamorphosis (% min–max)
Alteromonas spp.		+	12–87
Escherichia coli	+		6–93
Pseudomonas spp.	+		18–79
Staphylococcus aureus		+	4–75
Serratia marinorubra	+		14–56
Agar plate without bacteria			0
Petri dish (sterile)			0

crab-inhabited shells is not the result of a locally restricted metamorphic stimulus but rather the result of selection of animals already metamorphosed by environmental conditions including the hermit crab.

Induction of metamorphosis in *Aurelia aurita*

Around the island of Helgoland polyps of the scyphozoa *A. aurita* are found on rocks close to the shore (Janke 1986) while the medusae are often found in large cohorts floating in the open sea. In order to study their metamorphic induction we collected medusae-bearing planula larvae ready to be released. The larvae (several hundreds) were placed in large beakers containing filtered seawater and stones collected in the littoral. Within 1–2 days almost all larvae were found to have metamorphosed, but almost exclusively at the water–air interface by hanging with the oral surface downward, as also observed by Korn (1966). Simulating surf of the sea by vigorously bubbling air into the beaker we found the young polyps to fall to the bottom and to attach to the glass or to the stones by their sticky basal plate. One day after onset of bubbling only less than 5% of the several hundred polyps treated were still on the surface.

Discussion

Colonies of *Hydractinia echinata* were found on *Buccinum* spp. shells inhabited by hermite crabs (Schijfsma 1935) (in the North Sea around the island of Helgoland, Germany). We failed to find them on other substrates and on shells still inhabited by a snail. Thus, the specific biofilm covering shells inhabited by a crab was argued to induce metamorphosis (Müller 1969; Spindler and Müller 1972; Wittmann 1977; Leitz and Wagner 1993). However, all the materials we collected in the littoral and all marine bacteria strains caused metamorphosis of *H. echinata* efficiently when applied to an artificial substrate. It appears that the restricted abundance of *H. echinata* colonies does not reflect the restricted distribution of metamorphosis-inducing cues. Rather, selection following metamorphosis has to be envisaged to explain the observed distribution.

Shells of *Buccinum* spp. still inhabited by a snail were found to be covered with various organisms including small colonies of the hydrozoans *Laomedea flexuosa* and *Podocoryne carnea*. These animals were generally missing on shells inhabited by a crab. By studying the feeding behaviour of the hermit crab we were able to confirm well-known observations. The hermit crab scratches with the sharp terminal ends of its legs over the shell's surface and removes all it can. The colonies of *P. carnea* and *L. flexuosa* seem to be victims of this cleaning and feeding process. These animals adhere to the shell by thin tubes, termed stolons, which connect the polyps of the colony in a net-like manner. In contrast, *H. echinata* survives due to (at least) two properties: (1) they are firmly attached to the shell by a rigid confluent stolo-plate. Only when they are young do they grow by producing the fragile stolons. At that time a small colony may survive in one of the clefts the shell displays on its surface (Teitelbaum 1966). The clefts run orthogonally to the main movements of the crab's legs when scratching over the shell surface; (2) the adult colony produces strong spines (hence the name *H. echinata*), behind which the polyps retract when one of them is touched, thus preventing damage caused by the crab's legs. In addition, a colony of *H. echinata* living on a hermit crab's shell may get some of the crabs' food. In the laboratory a colony survives and reproduces excellently when exclusively fed with tissue fragments of molluscs, crabs and fish (unpublished experiments) which a crab produces when feeding on its prey. In summary, it appears that a crab disfavours the survival of several marine invertebrates, including the mentioned hydrozoa, on its shell as compared to *H. echinata*. In addition, *H. echinata* may profit from the crab. Therewith, the hermit crab strongly contributes to the restricted abundance of the colonies of *H. echinata*.

With respect to Aurelia aurita we argue that the contact of the anterior end of a larva to the water–air interface is sufficient to induce metamorphosis. Of course, the existence of a specific biofilm at the water–air interface can not be excluded. However, it may be possible that the interface simply acts as a physical barrier for substances to be released from the larva into the seawater or for substances to be taken up by the larva from the seawater. With respect to *H. echinata* it is suggested that metamophosis induction can be facilitated or even caused by reducing the export of the constantly produced NH_4^+ ions (Berking and Walther 1994). Exogeneously applied NH_4^+ ions induce metamorphosis (Berking 1988).

Korn (1966) found indications for positive phototactic behaviour of the larvae. Further, he observed metamorphosis of *A. aurita* to occur not only on stones and other substrates but also at the water–air interface but argued the latter to be an artefact, due to the calm water surface under laboratory conditions. We suggest that this behaviour is not an artefact but rather a rule. In *A. aurita*,

metamorphosis may take place in the open sea when mature larvae reach the surface of the water. The resulting young polyps, floating upside-down at the water surface, may be blown to the shore by wind where the surf causes them to lose contact with the surface and adhere to the underlying ground. When such a young polyp happens to attach to a suitable rock it will survive and may produce medusae later on. This may fit observations made in the field by Brewer (1978), who found most young polyps hanging with the oral surface downward from the lower site of stones. Larvae that happen to reach the rocks may undergo metamorphosis in the common sequence, starting with settlement and guided by specific cues presented by the various substrates, including those of already metamorphosed animals of the same species (Korn 1966). Field studies should be done to test our proposition deduced largely from experiments in the laboratory and isolated observations made in the field. If the view presented is largely correct, then in *A. aurita* there is not only a temporal but also a spatial separation of the two processes, metamorphosis and settlement. Further, and in contrast to the process in *H. echinata*, in *A. aurita* (final) settlement can follow metamorphosis.

With respect to several sedentary invertebrates, excellent experimental evidence indicates that species-specific cues cause the restricted and species-specific distribution of immobile adults (for review see Chia and Rice 1978; Clare et al. 1998). Selection, which follows metamorphosis, contributes in all cases to the observed differential distribution in the natural environment. With respect to *H. echinata* and *A. aurita*, the cues for metamorphosis induction appear to be much more widespread than the final distribution of the adults suggests. Regarding *H. echinata*, we would like to learn if one and the same metamorphosis-inducing agent is generated by all of the bacteria tested, and if so what its chemical nature is like.

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