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Larval development and settlement of a whale barnacle

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Larval development and settlement of whale barnacles have not previously been described, unlike intertidal barnacles. Indeed, the mechanisms of the association between barnacles and whales have not been studied. Here we describe the larval development and settlement of the whale barnacle, *Coronula diadema*, and possible involvement of a cue from the host in inducing larval settlement. Eight-cell stage embryos were collected from *C. diadema* on a stranded humpback whale, incubated in filtered seawater for 7 days, and nauplius larvae hatched out. When fed with *Chaetoceros gracilis*, the nauplii developed to stage VI, and finally metamorphosed to the cypris stage. The larval development looked similar to that of intertidal barnacles with planktotrophic larval stages. The cyprids did not settle in normal seawater, but did settle in polystyrene Petri dishes when incubated in seawater with a small piece of skin tissue from the host whale. This strongly suggests the involvement of a chemical cue from the host whale tissue to induce larval settlement.

Keywords: whale barnacle; epizoic barnacle; larval development; cypris larva; larval settlement; settlement cue

1. INTRODUCTION

Some barnacles are obligate commensals and are narrow in their selection of hosts. The hosts they associate with include a wide range of invertebrate and vertebrate taxa: sponges, corals, hydrozoans, molluscs, crustaceans, sea urchins, sea snakes, sea turtles and whales. Since Darwin described the structural modifications for life on large hosts in epizoic barnacles (Darwin 1854), their life cycles have been described for only a few species (Moyse 1961; Molenock & Gomez 1972; Lang 1979; Anderson 1994; Zardus & Hadfield 2004). In particular, larval development and settlement of whale barnacles have not previously been described. Moreover, the associations between the barnacles and whales have not been examined. In the present study, we succeeded in larval culture of a whale barnacle to the non-feeding cypris stage, and examined the larval settlement mechanism.

2. MATERIAL AND METHODS

(a) *Animals*

On 2nd January 2005, a humpback whale, *Megaptera novaeangliae* (body length: 8.4 m), was stranded on the coast of Chikura in Boso Peninsula, Chiba, Japan (34°56′ N, 139°57′ E). Several coronuline barnacles were observed on the whale, and one of them was collected from the pectoral fin with a piece of whale skin tissue (figure 1*a*) on 4th January. The barnacle was kept cool and transferred to the laboratory.

(b) *Larval culture*

On dissection of the barnacle body, we found a mass of embryos in the mantle cavity. The embryos were at the eight-cell stage (figure 1*b*), and were cultured in 0.22 µm filtered seawater (salinity: *ca* 36‰) at 20 °C with gentle aeration. After incubation for 7 days, several nauplius larvae hatched out from the mass of embryos (figure 1*c*). The hatched nauplii were cultured at 15, 20 and 25 °C with gentle aeration in 2 litre beakers in the presence of antibiotics, 20 µg ml^{-1} penicillin G and 30 µg ml^{-1} streptomycin. The larval density was 0.5 larva ml^{-1}. The nauplii were fed *Chaetoceros gracilis* at 4.0×10^5 cells ml^{-1}. The moulting sequence was tracked by placing individual larvae in 4 ml wells of 12-well culture plates at 20 °C. The light condition during the culture was 12L:12D.

(c) *Larval settlement assay*

Using the cypris larvae, we assayed larval settlement to test possible mechanisms of the association between the barnacle and the whale. As a control, 10 cyprids were incubated in 5 ml of normal filtered seawater at 20 °C in each polystyrene Petri dish with a diameter of 47 mm. To examine the effect of host tissue, 10 cyprids were incubated with a small piece (39 mg) of skin tissue from the host whale pretreated with 99.5% ethanol for 60 min. Effect of untreated fresh skin tissue of the host was also examined by the same assay. The light condition during the assay was 12L:12D.

3. RESULTS AND DISCUSSION

We identified the collected barnacle as *Coronula diadema* from morphological features (figure 1*a*). The barnacle had a firm anchorage and gripped the surrounding host tissue as described by Anderson (1994).

The moulting sequence was confirmed as passage through six nauplius stages to the cypris stage. The nauplius at stage I moulted to the stage II larva immediately after hatching. After 6 days at 25 °C, the larvae reached the non-feeding cypris stage after six moults. At 20 °C, the cyprids were detected after 7–8 days culture. At 15 °C, the larvae did not reach the cypris stage and finally died. Some larval stages are shown in figure 1. Although the morphological features of the larva in each stage were not significantly different from the well-known nauplius of *Balanus amphitrite*, there were some distinctive characteristics. The early nauplius, at stages II and III, has a pair of long frontolateral horns (figure 1*d*). The compound eyes in the late stage VI nauplius were peculiarly crescent-shaped (figure 1*f*). The cypris was long and narrow, and the head (apical region) was relatively flat (figure 1*g*). The compound eyes of the cyprid were spherical. There were many oil cells in the anterior region, as found in other cyprid species.

When cyprids were incubated in normal filtered seawater, no settlement was seen after several days. In contrast, out of 10 cyprids incubated with a small piece of skin tissue from the host whale pretreated with ethanol, four settled, and metamorphosed juveniles were observed on the plastic surface of the Petri dishes after 18 h (figure 1*h*). Untreated fresh skin tissue of the host was also an active inducer of larval settlement and metamorphosis; out of 20 cyprids, six settled and metamorphosed. All of the successfully settled larvae were found on the plastic surface of the Petri dish, not on the skin tissue. These results indicate that the cypris larvae of *C. diadema* were induced to settle and metamorphose by a cue from the host whale, but it seems that they can also settle on other substrata than the host skin after receiving the chemical cue. The settlement cue appears to be

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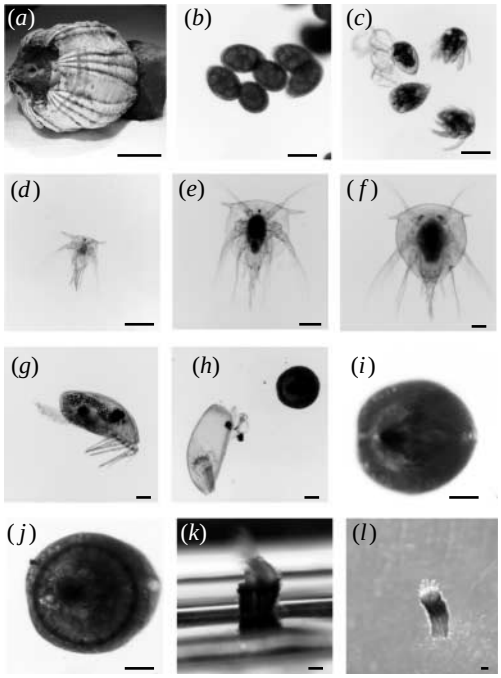

Figure 1. Adult, embryos, larvae and juveniles of the whale barnacle, *Colonula diadema*. (*a*) Adult barnacle with a small piece of whale skin. (*b*) Mass of embryos at eight-cell stage. (*c*) Hatching nauplii (day 0). (*d*) Nauplius II (day 0). (*e*) Nauplius IV (day 4). (*f*) Nauplius VI (day 6). (*g*) Cyprid (day 7). (*h*) Juvenile just after metamorphosis from cyprid (day 12). (*i*) Juvenile (day 14). (*j*) Base of juvenile (day 14). (*k*) Juvenile (day 15). (*l*) Juvenile (day 18). (*a*) Scale bar, 10 mm; (*b–l*) scale bars, 100 μm.

released from the tissue of the host whale, but we do not know whether the cue is active when adsorbed on the substratum or when dissolved in seawater. The released cue may be adsorbed onto the Petri dish surface. Although the nature of the settlement cue is unknown, it is stable when treated with ethanol for 60 min. In the intertidal barnacle, *B. amphitrite*, larval settlement is induced by a protein complex (SIPC) isolated from conspecific adults, and this protein is thought to be involved in the phenomenon of gregarious settlement (Matsumura *et al.* 1998*a*,*b*; Kato-Yoshinaga *et al.* 2000; Clare & Matsumura 2000). Recently, homology between SIPC and proteins belonging to alpha-2-macroglobulin super family was suggested by cDNA cloning (Dreanno *et al.* 2004). Alpha-2-macroglobulin is widely found in vertebrates, including whales. It is possible that the whale macroglobulin or related proteins function as settlement inducers for the specific barnacle. However, more information is necessary to clarify the settlement-inducing mechanism of whale barnacles.

The settled larvae moulted into juveniles (figure 1*h–l*). The process of metamorphosis was similar to that in other barnacle species. However, some specific morphological features were observed after metamorphosis. After 2 days, a ring-shaped structure with 18 short spine-like processes on the inside was observed at the base of the juvenile (figure 1*j*). The structure seemed to hold onto the substratum, and to develop into a firm anchor that could grip the surrounding host tissue. Growth of the juvenile on the polystyrene surface was distinctive; the juvenile barnacle grew mainly upward and showed a cylindrical shape (figure 1*k*,*l*). In the cylindrical juvenile, distinct wall plates were not observed, but several stripes were shown on the outside of the juvenile. It is not certain that the cylindrical shape is the normal morphology of the young barnacle in nature on the whale skin. However, it is interesting that the cylindrical shape is similar to the adult shape of another whale barnacle species, *Tubicinella major*, which is found on the right whale and is almost entirely embedded in the host skin (Scarff 1986). After settlement, the juveniles were fed laboratory-reared diatom, *C. gracilis*, and brine shrimp, *Artemia* sp. However, their excrements were not observed, and the cylindrical-shaped juveniles of *C. diadema* on the polystyrene surface finally died in the laboratory one to two weeks after settlement.

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