An Ancient Chemosensory Mechanism Brings New Life to Coral Reefs

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The first scleractinians, progenitors of modern corals, began to appear 240 million years ago; by the late Jurassic (150 Ma) most families of modern corals had evolved and begun forming reefs (1, 2). Mechanisms controlling the recruitment of new corals to sustain these structures are, however, poorly understood (3). Corals, like many marine invertebrates, begin life as soft-bodied larvae that are dispersed in the plankton (3, 4). As the first step in developing a calcified coral colon, the larva must settle out of the plankton onto a suitable substratum and metamorphose to the single calcified polyp stage cemented to the reef (3, 5). Our analyses of the metamorphic requirements of larvae in divergent coral families surprised us by revealing the existence of a common chemosensory mechanism that is required to bring larvae out of the plankton and onto the reef. This mechanism appears to be quite old, predating both the phylogenetic divergence of these coral families and the development of different triodes of coral reproductive strategies.

We analyzed the requirements for metamorphosis of larvae from 10 species of Pacific Acropora—the acroporid genus with by far the greatest number of known species (76 in the Indo-Pacific, about one-sixth the estimated number of scleractinian species in that region)—and three species representing three common genera of Pacific Faviidae, the second most speciose scleractinian family (1, 2). Most, but not all, species of Acropora and Pavia (genera that first appear in the fossil record in the Eocene and Cretaceous, respectively) are widely distrib-
Figure 1. Pacific acroporid larvae exhibit a stringent requirement for contact with crustose red algae for metamorphosis; development and decay of metamorphic competence is dependent on larval age. (A) Metamorphosis of *Acropora nasuta* larvae (3, 5 and 7 days post-fertilization) in response to various crustose red algae and controls after 48 h incubation in 10 ml filtered seawater (FSW). Algae: 1 = 0.22-Am FSW alone (control); 2 = Lobophora variegata (brown alga, control); 3 = Perssonella sp.; 4 = *Hydrodolithon reinboldii* (form i); 5 = flydololithon reinboldi (form ii); 6 = *H. drololithon reinboldii* (form iii); 7 = *H. drololithon reinboldii* (form iv). Values are mean % (± 1 std. error) metamorphosis of five individuals in each of two trials (n = 2). Percentages were arcsine transformed for analysis. (B) Metamorphic response of *Acropora digitifera* larvae (3, 5 and 7 days post-fertilization) to algae and controls, as in (A). (C) Metamorphosis of *A. nasuta* larvae after a 48-h incubation with *Perssonella* sp. (U) or a parallel exposure to FSW alone (O). Individual points represent ages (days post-fertilization) at challenge: metamorphosis (%) ± mean; error bars = s.e. of n = 2 (days 3–7) or 3 (days > 7). (D) *A. diguilla* larval response as for (C). (E) *A. tennis* larval response as for (C), n = 3 all days. Larvae of each species were obtained as follows: The maturity of coral gonads was confirmed (14); colonies were transferred from the reef to laboratory aquaria and kept in darkness until the gamete clusters were ready to be released; the colonies were transferred to individual containers for spawning; gamete clusters from a single colony were collected and dispersed by sequential transfer into FSW; eggs and sperm were separated for immediate cross-fertilization with gametes of other colonies (> 95% fertilization rate); all spawnings (1/species) produced 5-10,000 larvae/batch; each batch was cultured in 3 I FSW (changed daily) at 26°C for 30 d; larvae of each species were held in these rearing chambers until randomly selected for assay at the ages indicated. For assays, fragments were
ALGAL CUE FOR CORAL METAMORPHOSIS

The stringency of this requirement (no metamorphosis in the absence of an exogenous cue) and the specificity (dependence on crustose red algae) of cue recognition persist for the duration of larval competence for metamorphosis (Fig. 1C, D, E). Species-specific differences in the duration of competence are obvious. The magnitude of the larval response for each species is dependent upon larval age (Fig. 1). Larvae exhibited little or no response to Peyssonnelia sp. at 3 days post-fertilization; responsiveness developed by 5 days, peaked at 7 days, and declined thereafter at rates that are species-specific (Fig. 1C, D, E).

If these results reflect larval potential for dispersal in the plankton, then the differences in the rates of decline of competence among all of the tested acroporid species, with no accompanying loss in stringency or specificity of cue requirement (e.g., Fig. 1), suggest species-specific differences in the windows of opportunity for successful settlement and metamorphosis on the reef. Assuming that rate of decrease of competence with larval age is inversely correlated with the distance of dispersal from parental colonies (all other chemical and hydrodynamic factors being equal), A. tenuis appears to have the potential for widest dispersal and significant recruitment for at least 5—30 days post-fertilization. A. digitifera appears to have the potential for somewhat wider dispersal than A. nasuta, although the majority of potential recruits from both species will be dispersed over similar distances (5—12 days). Although the bulk of settlement and metamorphosis might occur soon after the larvae reach competence, these differences among species that recognize the same algae may serve to decrease post-settlement competition for space among some of the settlers. Variation among these species in timing of gamete release during mass-spawning events (11-14), in concert with variations in currents and other hydrodynamic factors, may additionally contribute to a reduction in the potential for post-settlement interaction.

Significantly, we believe, larvae of these Pacific acroporids and those in two Caribbean genera, Agaricia and Leptoseris (Agariciidae), both appear to recognize and require the same class of algal cue for the induction of larval settlement and metamorphosis. This is in spite of their very different modes of sexual reproduction and larval development (3). Acroporids participate in mass-spawning events during which millions of gametes are released into the plankton for cross-fertilization, followed by larval development in the plankton; in contrast, agaricids have evolved the less common mode of

prepared from algae as previously described (6, 7, 9); live H. reinholdii specimens were grouped according to the surface characteristics of different growth forms (by A. N. C. M.): specimens preserved in formalin and seawater were dehydrated, embedded in paraffin, sectioned (8-μm thickness), and identified with a light microscope to species and genus level (by M. B.)—Lobophora variegata (Lamouroux), H._rdrefithen reinholdii (Weber van Bosse et Foslie) Foslie, Perssonelloja sp. [similar to P. rhscura Weber van Bosse and P. conchicola Piccone et Grunow (19)].

Larvae maintained in seawater alone (≥30 days) continue planktonic swimming and never develop beyond the larval stage illustrated in Fig. 2A (Fig. 1C, D, E). Metamorphosis is initiated on contact with an inductive alga, e.g., Peyssonnelia sp. or any of four morphological forms of *Hydrolithon reinholdii*; larvae rapidly stop swimming, their bodies elongate and remain in close contact with the algal surface, and within a few hours, they round up and cement themselves to the algal surface or adjacent substratum. The final stages of metamorphosis are marked by the development of 12 radial skeletal elements, the calcified septa and costae (Fig. 2B). Larvae of the mass-spawning congener *A. tenuis, A. Florida, A. geinmifera, A. Formosa, A. hyacinthus*, A. sp. 1, A. sp. 4 and A. sp. 5 all exhibit a similar strict requirement for contact with either or both Peyssonnelia sp. and H. reinholdii (Fig. 2C; and A. N. C. Morse et al., University of California, Santa Barbara, in prep). Although, in some instances, the brown alga *Lobophora variegata* promoted first stage elongation of the larvae, further development rarely if ever occurred. When larvae from seven acroporid species (50 larvae/species incubated together) were given a choice between crustose red algae and brown algae, all that had metamorphosed after 4-h exposure (70%) were found only on the crustose red algae (Peyssonnelia sp. and H. reinholdii) (Fig. 2C); no larval metamorphosis was detected on brown algae or on the chamber surfaces; control larvae (same number and species) remained swimming. In a similar experiment, 500 competent larvae from each of five acroporid species (*A. nasuta. A. tenuis, A. Formosa, A. hyacinthus, and A. geinmifera*, tested together in a 30-1 flow-through tank) were presented a choice of algae, live corals, inert substrata collected from the reef, "fouled" panels, and a variety of inert materials commonly used as settlement plates; all settlement and metamorphosis occurred only on coralline algae (A. N. C. Morse et al., University of California, Santa Barbara, in prep).

The stringency of this requirement (no metamorphosis in the absence of an exogenous cue) and the specificity

OVA; coral *algae interaction: F = 0.89, df = 4, P = -0.503), *A. digitifera* exhibited less variation in response among algae (one-way ANOVA: F = 0.61, df = 6, P = 0.72) than did *A. nasuta* (one-way ANOVA: F = 2.9, df = 6, P = 0.095). Such patterns are similar to the species-specific differences previously found in agaricid corals (10). Morse

found only on the crustose red algae
Figure 2. Larval behavior and early metamorphic changes of Pacific acroporid corals in response to a morphogenic cue associated with Pacific crustose red algae. (A) Typical shape of soft-bodied larva ? 3 days post-fertilization: _A. digiulera_ larva, 8 days post-fertilization, swimming normally in the water column. (B) Final stage of metamorphosis; formation of radial skeletal elements (septa and costae) and elevation of central area around mouth: _A. nasula_ larva, 8 days post-fertilization. incubated with _Peyssonelia_ sp. (C) Early-stage metamorphosis (4 h) of a mixture of larvae of _A. nasula, A. digiulera, A. tenantis, A. Formosa, A. gemmu%era, A. jerrydae, A. sp. 5_ on whole specimens of _H. reinboldii_ and _Peyssonelia_ sp. (D) Metamorphosis of _A. jerrydae_ larvae in response to inductive molecules purified from _H. reinboldii_ and coupled to resin beads. In (A) and (B) the assays were as described in Fig. 1. In (C) 20 competent larvae of each of seven species were incubated together in 200 ml FSW with or without small intact specimens of _Peyssonelia_ sp., _Hdrolithon reinboldii_ and _Lobophrora varfegaza_ in (D) the resin with adsorbed inducer was the same preparation as that assayed in Table I; larvae were batchmates of those in (C).

sexual reproduction in which cross-fertilization occurs internally, and subsequent larval development and brooding occur within the maternal polyps.

The possibility of similar cue recognition in members of these two families was first suggested by the fact that the algal species (_Hdrolithon reinboldii_ and _Peyssonnelia_) that are shown here to induce metamorphosis of Pacific acroporid larvae have congeners in the Caribbean that induce metamorphosis of agariciid larvae (5-10). This suggestion is confirmed by the demonstration (Table I) that the Pacific alga, _Hdrolithon reinboldii_, contains an extractable polymeric morphogen that is apparently in the same class of molecules as one previously identified in its Caribbean congener, _H. boergesenii_. Fragments of both the Caribbean and Pacific algal congeners (_Hdrolithon_ and _Peyssonnelia_) induced levels of metamorphosis in _Agaricia hnnulis_ larvae that are not significantly different (Table I; one-way ANOVA: $F = 1.59, df = 4, P = 0.198$). Moreover, when the same procedures developed for biochemical purification, characterization, and coupling (7, 9, 15) of the polymeric inducer of agariciid larvae from _H. boergesenii_ to a hydrophobic resin were applied to _H. reinboldii_, the purified molecule adsorbed to resin was recognized by _A. hnnulis_ larvae (Table 1). Larval settlement and metamorphosis of at least seven species in two genera of Caribbean agriciids is strictly dependent on chemosensory recognition of a unique class of sulfated glycosaminogly-
can (7, 9) that is associated with the cell walls of a number of Caribbean crustose red algae (5, 7-9, 15). Enzymatic and biochemical analyses demonstrated that this inductive polymer has a molecular weight of 5-10 Kd and does not induce metamorphosis in coral larvae that are not induced by the intact parental algae (7). Both the biochemical characterization of the inducer isolated from an inductive Pacific alga and its recognition by A. humilis larvae indicate that this morphogen belongs to the same class of algal cell-wall polysaccharide as the compound obtained from Caribbean red algae. As we show here, acroporid larvae assayed at Akajima metamorphosed in response to both *H. reinboldii* fragments and intact algae (Figs. 1, 2B, C), as well as to this same batch of resin-adsorbed morphogen purified from *H. reinboldii* (Fig. 2D) when incubated with resin lacking the adsorbed molecule, larvae of the Pacific corals remained arrested with no metamorphosis. Settlement and metamorphosis in the tested *Acropora* species are thus apparently induced by chemosensory recognition of the same class of algal sulfated glycosaminoglycan.

The acroporids and agariciids are members of the recently defined Glade of complex scleractinians (16). In experiments otherwise identical to those above, larvae of the mass-spawning faviid corals from Akajima—*Favia Javus*, *Goniastrea retiformis*, and *Cyphastrea* sp.—members of a taxonomically distinct Glade of robust corals (2, 16, 17), also exhibited a strict requirement for the same class of algal morphogen recognized by the complex coral larvae. Thus, the inducer molecule purified from the two Pacific algae, *Hydrolithon reinboldii* and *Peyssonnelia* sp. (each adsorbed to separate batches of hydrophobic resin) induced metamorphosis of *Cyphastrea* sp. larvae. There was no spontaneous metamorphosis in the presence of resin with no adsorbed cue. Fragments of the intact alga *H. reinboldii* induced metamorphosis of *Cyphastrea* sp., whereas seawater alone, dead coral branches, and the brown alga *L. variegata* produced no metamorphic response. Similarly, *Favia favus* and *Goniastrea retiformis* metamorphosed in response to fragments of intact *Peyssonnelia* sp., but were unresponsive to *L. variegata* and seawater alone. Relatively few of these faviid larvae were available, which limited the testing of the other algae and purified inducers adsorbed to resin with each coral species. The results, however, demonstrate the stringency and specificity of the requirement of these robust corals for the same class of algal inducer (A. N. C. Morse et al., University of California, Santa Barbara, in prep.).

The algal-cue-dependent settlement and metamorphosis of agariciids described here has been shown to operate effectively in the ocean as well as in the laboratory (9), and to contribute to substratum specificity of agariciid recruitment in the natural environment (6). We further suggest that this mechanism may enhance the probability of successful reproduction of coral species that depend on cross-fertilization. In mass-spawning corals the success of cross-fertilization of gametes in the plankton depends on a rapid encounter with gametes from another colony of the same species, and thus on the propinquity of reproductive colonies of the same species. Similarly, species with internal cross-fertilization depend on the transfer of sperm from one colony to another. Field manipulation of colony distances in *Agaricia humilis* reveals that colonies must be <2 m from their nearest neighbor for successful production of larvae (P. T. Raimondi and A. N. C. Morse, University of California, Santa Barbara, in prep.).

The evidence presented here indicates that representa-


