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Tissue compatibility between colonies and between newly settled larvae of *Pocillopora damicornis*

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Abstract. Grafting experiments with newly settled larvae and with adult colonies of *Pocillopora damicornis* were performed. When pairs of newly settled larvae released from different colonies were kept in contact, they fused to form an aggregated colony. Even newly settled larvae derived from colonies belonging to different color morphs fused with each other and no sign of allogeneic rejection was observed. However, when branches of adult colonies belonging to different color morphs were kept in contact, they did not fuse. Fusion was observed only when branches derived from the same colony were paired. The present results suggest that juvenile corals lack the functional histocompatibility system as shown by adult colonies.

Many workers have reported that, in scleractinian corals, two pieces from the same colony (isografts) fuse when kept in contact, while intercolony grafts (allografts) are incompatible (Hildemann et al. 1975, 1977; Potts 1976, 1978; Rinkevich and Loya 1983). If two colonies are compatible, they are assumed to have the same genetic composition and hence to be asexually produced from the same colony. Thus intercolonial grafting within the same species provides a tool for assessing genetic diversity of corals and the relative importance of sexual and asexual reproduction in reef-coral populations (Bothwell 1981; Bak and Criens 1982; Jokiel et al. 1983; Neigel and Avise 1983).

Early workers reported that, when planulae which settle close to one another grow and come into contact, they fuse to form an aggregated colony (Stephenson 1931; Atoda 1947a, b, 1951a, b). Sammarco (1982) observed natural fusion of juvenile corals in the reef. Planulae of corals are generally believed to be produced sexually, though in *Pocillopora damicornis*, asexual production of planulae has been suggested (Stoddart 1983, 1984). It is interesting that these newly settled planulae do not reject each other by an allogeneic histoincompatibility response. One possibility is that juvenile corals have not yet developed a histocompatibility system as shown by adult colonies. Another possibility is that a coral does not reject its siblings or other conspecific corals that are genetically close to itself. Recently Rinkevich and Loya (1983) reported that, even when allografts appear to fuse with each other, a narrow gap exists between the two tissues when observed with a scanning electron microscope. Thus, it is also possible that no true fusion occurs in an aggregated colony. It seems necessary to investigate tissue compatibility between primary polyps or newly settled larvae of the same species in more detail.

In this paper, tissue compatibility between newly settled larvae derived from the same colony or from different colonies of *Pocillopora damicornis* (Linnaeus) was examined, as well as tissue compatibility between adult colonies. It will be shown that newly settled larvae from the same colony or even from different colonies fused with each other to form an aggregated colony. In adult colonies, fusion was observed only in isografts. These observations strongly suggest that corals lack a functional histocompatibility system in the early stages of development.

Materials and methods

Collection of animals

Colonies of *P. damicornis* about 15 cm in diameter were collected from the reef in Kaneohe Bay, Hawaii and were transported into holding tanks without exposing the animals to air.

There are two color morphs, "Y" and "B" types, of *P. damicornis* in the reef of Kaneohe Bay (Richmond and Jokiel 1984). The "Y" type colonies were yellowish brown with yellow branch tips and had stouter branches. The "B" type colonies were brown, with white branch tips and had more fragile branches. These two morphs differ not only in color and growth form but also in the timing of planula release. As colonies belonging to these two morphs may occur side by side, their differences are unlikely to be the result of environment and are probably genetic. In this experiment two colonies belonging to different color morphs were paired in most of the intercolony graftings to make certain that two colonies paired were genetically different (allogeneic).

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Grafting experiments with adult colonies

Using forceps, branches about 5 cm long were removed from each colony. Two branches from the same colony or from different colonies were tied to a plastic petri dish with thread so that their tips touched each other. Four replicated pairs were made in each combination. Paired branches were reared in holding tanks supplied with running sea water for 32 or 50 days. They were observed under a dissecting microscope at intervals of 1–2 weeks.

In *P. damicornis*, it was difficult to make good contact since colonies of *P. damicornis* are highly branched and have rough surfaces. It is possible that tissue fusion did not occur because of insufficient tissue contact. To exclude this possibility, the paired branches were detached at the end of the contact experiment and the surface of the branches at the contact point was examined. Valid pairings showed exposed skeleton at the contact point indicating continuous contact between coenosarc tissues of both branches.

When tissues appeared to be fused, a gentle mechanical stimulus was applied to a polyp close to the interface to determine if the excitation was propagated through the interface, causing retraction of the polyps of the other branch.

Grafting experiments with newly settled larvae

Planulae of *P. damicornis* were collected by the method of Richmond and Jokiel (1984). Colonies of *P. damicornis* were placed in containers (2.5 l) supplied with running sea water. Overflowing water was drained through collectors made from polyvinyl chloride (PVC) pipe, the base of which was covered with 180 μm plankton netting. The lateral inner surface of the collector was covered with plastic sheet. The surface of the sheet was made rough by rubbing with sandpaper. Usually most of the planulae settled on the plastic sheet within a few days after being released.

When settled planulae became flattened and began to secrete a skeleton, a piece of plastic sheet with the settled planula was cut off from the lateral wall of the collector. Two pieces of plastic sheet, each with one primary polyp on it, were held on a glass slide so that the primary polyps abutted (Fig. 3 A). Primary polyps derived from different colonies, usually of the different color morphs, were paired. The paired polyps were maintained in a chamber made of PVC pipe, the base of which was covered with 300 μm plankton netting. The chambers were immersed in a holding tank and were aerated. The paired primary polyps were observed at intervals of 2–4 days under a dissecting microscope. Filamentous algae were removed with forceps or pipette at each observation. Some specimens were examined under a light microscope and photomicrographs were taken at 32 or 63X. A gentle mechanical stimuli was applied on one of the paired polyp to examine whether the excitation was propagated through the interface causing retraction of the adjacent polyp. Although primary polyps began to bud and possessed up to six buds at the end of the experiment, these polyps with rudimentary buds are still called primary polyps or newly settled larvae.

Planulae released by a colony have a tendency to settle in aggregates just below the surface of sea water as reported previously (Stephenson 1931). Plastic sheets each with one to several aggregates of primary polyps on it were cut off and were maintained in the manner just described.

Results

Tissue compatibility between adult colonies

The branches of colonies belonging to different color morphs (allografts) did not fuse with each other (Table 1). Fusion was observed only in the pairs of branches which were derived from the same colony (isografts). In this experiment, only two combinations of colonies belonging to the same color morphs were examined. Tissue fusion was not observed in this case (Table 1; B21–B22, Y24–Y25).

Fusion or nonfusion of tissue could be definitely recognized under a dissecting microscope in most pairs of *P. damicornis* colonies 32 or 50 days after grafting. When fusion occurred, the tissue appeared continuous with no sign of a junction and zooxanthellae were uniformly distributed in the region of contact as in normal tissue (Fig. 1 A). When a gentle mechanical stimulus was applied to a polyp close to the interface between two branches, retraction of polyps occurred on both sides of the interface. When tissues were not fused, they were separated by a white line which might represent two layers of ectoderm lacking zooxanthellae (Fig. 1 B). Sometimes, the skeleton in the narrow region close to the contact

Table 1. Tissue compatibility between adult colonies of *Pocillopora damicornis*. Numbers of fused, not fused, and apparently fused pairs are shown. Number of contact points in each category is also shown, since some pairs had more than one contact point. The letter indicates the color morph and the numbers following the letter each designate the colony number. B, brown with white branch tips. Y, yellowish brown with yellow branch tips

Colony pairs	Fusion		Apparent fusion		Nonfusion		Duration of contact (days)
	Pairs	Contact points	Pairs	Contact points	Pairs	Contact points	
Syngeneic pairs							
B13–B13	4	5	0	0	0	0	50
B22–B22	3	5	0	0	0	0	32
Y11–Y11	2	3	0	0	0	0	50
Y12–Y12	2	2	0	0	0	0	50
Y24–Y24	4	5	0	0	0	0	32
Allogeneic pairs							
B13–Y11	0	0	0	0	1	3	50
B13–Y12	0	0	0	0	1	1	50
B21–Y24	0	0	0	0	2	3	32
B22–Y25	0	0	1	1	2	3	32
B23–Y26	0	0	2	2	3	4	32
B21–B22	0	0	1	1	0	0	32
Y24–Y25	0	0	0	0	2	5	32

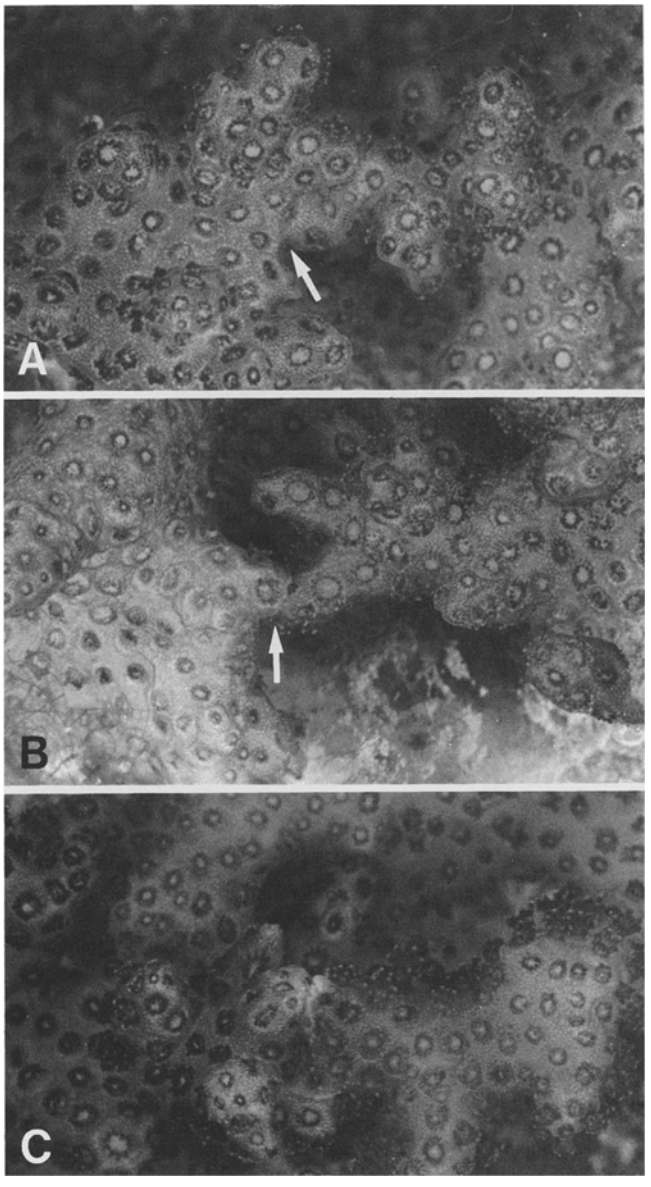

Fig. 1 A–C. Typical outcomes of grafting experiments between adult colonies of *Pocillopora damicornis* (X4). **A** Isograft showing compatible fusion at interface (arrow). **B** Allograft showing a white border line (arrow) at interface which indicates incompatibility. This pair consisted of branches of Y11 (left) and B13 (right) colonies and was maintained for 48 days. **C** Allograft showing cytotoxic incompatibility. This pair consisted of branches of B21 (left) and Y24 (right) colonies and was maintained for 32 days. Note blanching and tissue death of the right branch at contact zone

point was exposed in one branch of the pair (Fig. 1 C). In addition, the retraction of polyps did not occur on both sides of the interface. However, when the branches of the nonfused pair were detached, tissue bridges connecting two branches were frequently observed at the site of contact. This may indicate that the tissue of one branch was attached to the exposed skeleton of the other branch. Sometimes a cylindrical tissue bridge was observed when the paired branches were slowly detached. Mesenterial filaments were frequently observed in such a cylindrical tissue bridge.

There were, however, some cases in which the tissues of allogeneic colonies were apparently fused. In these cases, however, the distribution of zooxanthellae was not uniform. The region at the interface was white with less zooxanthellae than on other parts of the branch. No propagation of polyp retraction was observed in apparently fused pairs. When the branches were detached, there was a tissue bridge similar to that described for nonfused pairs. A small thin skeletal ridge was frequently observed at the contact surface indicating that secretion of the skeleton occurred at the interface of the branches. Both nonfusion and apparent fusion were frequently observed at different contact points of the same pair. It seems that nonfusion and apparent fusion are not different responses. In apparent fusion, it is likely that the tissue of one branch grows over the exposed skeleton of the other branch. However, the tissues of the two branches might remain discontinuous with a fine gap between them.

Tissue compatibility between newly settled larvae

Planulae released from the same parent colony often settle in aggregates. At first these planulae were independent and attached to one another with their aboral surfaces touching each other. They fused to form an aggregated colony in a few days (Fig. 2 A, B). Calyces did not fuse but were separated by an intervening skeleton. When a gentle mechanical stimulus was applied to one polyp, retraction of the neighboring polyp also occurred. The propagation of polyp retraction was observed in 29 out of 49 aggregated colonies within 3–8 days after settlement. Polyps of other aggregated colonies were contracted or not sensitive to mechanical stimuli and it was difficult to determine whether propagation of polyp retraction occurred or not.

When two planulae released by the same colony settled close to each other, they also fused when they came into contact (Fig. 2 C, D). In this case, the skeleton between two calyces was smooth as is the coenosteum in a normal colony.

When newly settled larvae derived from different parent colonies were paired, they came into contact as they grew and then fused (Fig. 3). Even settled larvae derived from colonies belonging to different color morphs fused (Table 2). Zooxanthellae were distributed uniformly in the interface zone as in other regions of the polyp (Fig. 3 D).

Propagation of polyp retraction through the interface was sometimes observed (Table 2). In some other cases, when one polyp retracted in response to a mechanical stimulus, the other polyp extended. This may indicate that the coelenteron of both polyps is continuous and that these polyps are functionally united, although fusion of the coelenteron was not confirmed histologically in this study. In this experiment, the observation was made 11–15 days after the setting of the polyp pairs. No sign of allogeneic rejection was observed during this period.

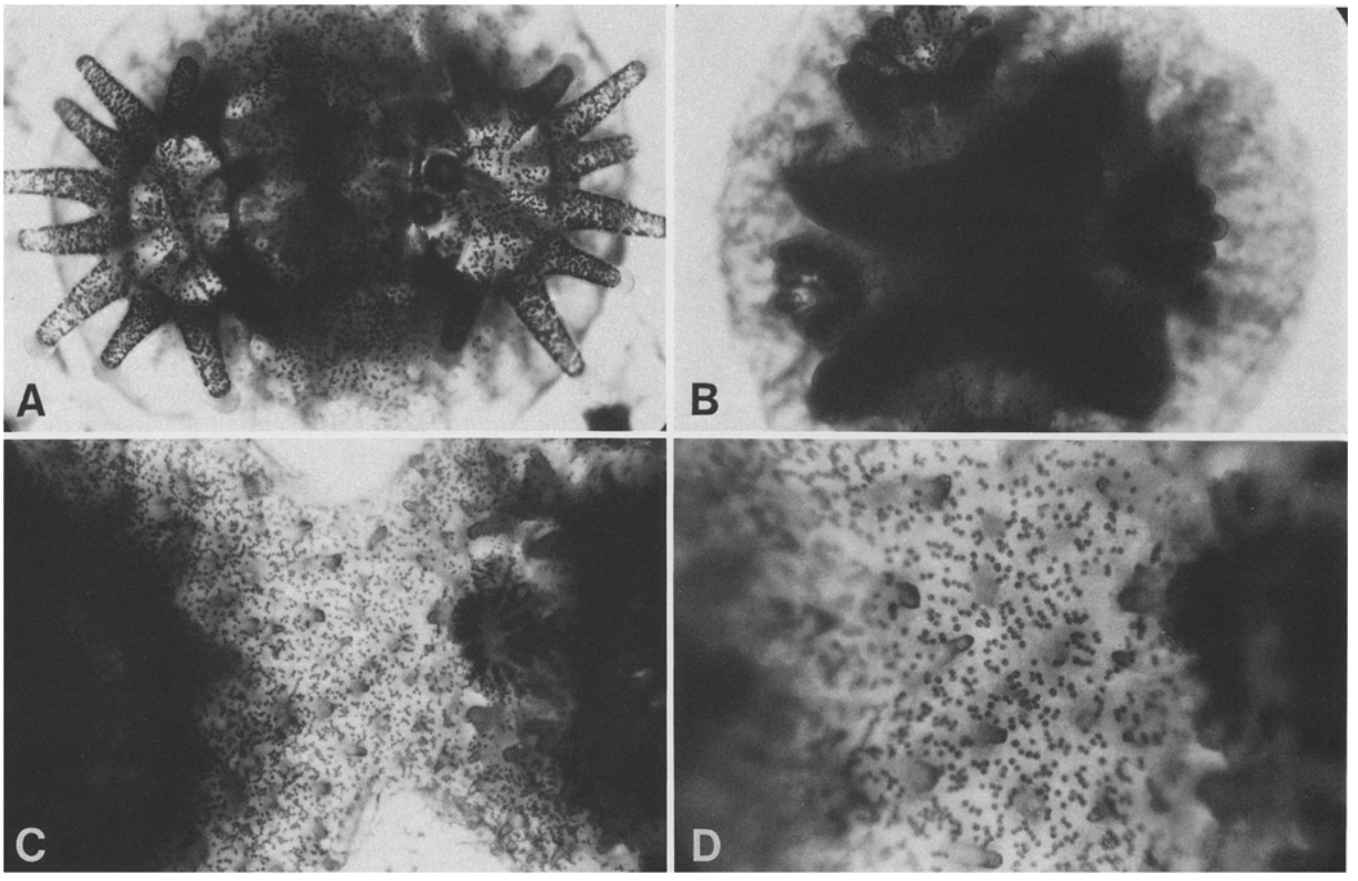

Fig. 2 A–D. Fusion of newly settled larvae derived from the same parent colony of *Pocillopora damicornis*. **A** Two newly settled planulae fused with their aboral surfaces touching each other. **B** Four settled planulae fused with their aboral surfaces touching one another. **C** Two planulae settled close to each other fused when they came into contact. Tissues and skeletons fused smoothly with no sign of junction. **D** Interface zone of C observed at a higher magnification. Note uniform distribution of zooxanthellae. Magnification, X40 in A, B, and C, X79 in D

Table 2. Tissue compatibility between newly settled larvae derived from different colonies of *Pocillopora damicornis*. Numbers of fused pairs and of pairs which showed propagation of polyp retraction are shown. Number of pairs which failed to make contact with each other during the observation period (11–15 days) and mortality of the paired polyps are also shown

Source colonies of paired primary polyp	Fused pairs	Pairs that showed propagation of polyp retraction	Pairs that failed to make contact	Mortality of paired polyps (%)
B1–Y1	11	6	12	12.1
B3–Y1	2	0	4	60.0
B2–Y1	3	2	1	25.0
B1–Y2	3	0	4	22.2
B1–B3	3	1	0	0.0
Y1–Y2	0	0	2	33.3

A preliminary electrophoretic study was performed to test whether primary polyps, or parent colonies, used in this grafting experiment were genotypically different from one another. Three isozymes (leucyl glycylglycine peptidase, phophoglucomutase, and phosphoglucose isomerase) were examined according to the method described in Stoddart (1983, 1984). Five colonies (Y1, Y2, B1, B2, B3) used for sources of planulae in the grafting experiment displayed four different isozyme patterns; B1 and B2 colonies showed the same isozyme pattern. Fused primary polyps were separated with a razor blade and each polyp was separately treated for the electrophoretic study. In this preliminary electrophoretic study, only three pairs were examined. These pairs consisted of primary polyps derived from Y1 and B1 colonies or Y1 and B2 colonies. In both cases, paired polyps displayed different isozyme patterns from each other. Each polyp, however, displayed the same electrophoretic pattern of isozymes as its parent colony as described previously by Stoddart (1983). This indicates that primary polyps or newly settled larvae which are genotypically different from each other can fuse.

Discussion

The present grafting experiment with adult colonies of *P. damicornis* shows that isografts are invariably compatible, while allografts are incompatible. This is consistent with previous studies on tissue compatibility of other corals (Hildemann et al. 1975, 1977; Potts 1976, 1978; Bak and Criens 1982; Jokiel et al. 1983; Rinkevich and

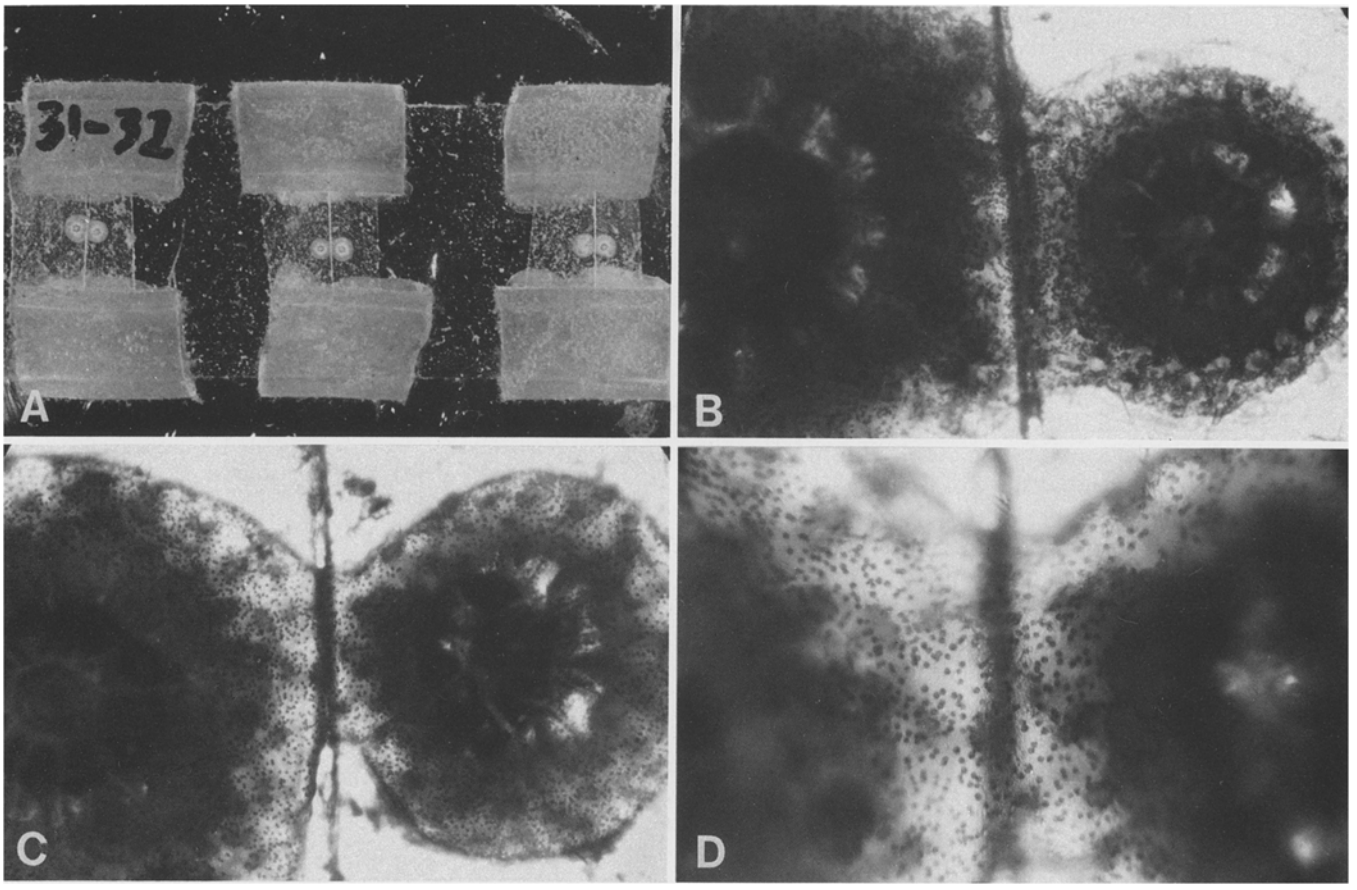

Fig. 3 A–D. Fusion of newly settled larvae derived from different colonies of *Pocillopora damicornis*. **A** Fused primary polyps showing technique of grafting primary polyps (X3.0). Plastic sheets mounting primary polyps were held on a slide glass so that paired polyps can be placed side by side. **B** Fusion of newly settled larvae derived from Y1 (left) and B1 (right) colonies. This pair was photographed 10 days after the setting. **C** Fusion of newly settled larvae derived from Y1 (left) and B2 (right) colonies. This pair was photographed 13 days after the setting. **D** Interface zone of C observed at a higher magnification. Note uniform distribution of zooxanthellae. The dark line in B, C, and D represents the edge of the plastic sheet. Magnification, X40 in B and C, X79 in D

Loya 1983; Neigel and Avise 1983; Hidaka and Yamazato 1984).

Though apparent fusion was observed in allogeneic pairs of *P. damicornis*, this can be distinguished from true fusion by the following points. The retraction of polyps does not propagate through the interface between two branches in apparently fused pairs. Zooxanthellae are distributed irregularly at the interface of apparently fuse branches, contrary to the uniform distribution in syngeneic pairs. Furthermore apparent fusion and nonfusion were frequently observed in different sites in the same pair of colonies. Apparently fused tissues may well be separated by a fine gap which is not discernible under a stereomicroscope. Rinkevich and Loya (1983) reported that apparently fused tissue of allografts of *Stylophora pistillata* was separated by a fine gap when observed with a scanning electron microscope. Bak and Criens (1982) stated that decalcification always proved the tissue to be discontinuous even no suture could be seen between the adjoined tissues of the two allogeneic colonies of *Acropora palmata*. Allogeneic rejection in *P. damicornis* also appear to be relatively mild, though in some cases cytotoxicity was observed.

The present results show that newly settled larvae produced by genotypically different colonies can fuse with each other. Since adult colonies of "B" and "Y" types are incompatible, this strongly suggests that juvenile corals lack the histocompatibility system as shown by adult colonies.

Early workers reported that planulae settling close to each other fuse to form an aggregated colony (e.g., *Pocillopora bulbosa* and *Porites haddoni*, Stephenson 1931; *Pocillopora damicornis*, Atoda 1947a; *Stylophora pistillata*, Atoda 1947b; *Galaxea aspera*, Atoda 1951a; *Seriatopora hystrix*, Atoda 1951b). However, in these early studies, it was not clearly described whether the planulae are from the same parent colony or from different colonies. Even if the planulae were from different colonies, it is not known whether primary polyps forming an aggregated colony were derived from a single colony or from a mixed population of planulae produced by different colonies. This is the first study that proves that primary polyps or newly settled larvae derived from genotypically different colonies can fuse with each other.

The idea that young animals lack an immunological system is not uncommon. Immunological incompetence

of foetus of mammals and amphibian larvae is well known (Cooper 1976). If an animal is grafted with allogeneic tissue at a period when its immunological system has not matured, it will not reject the grafted tissue, but instead, may develop tolerance.

It is not known if the colony formed by the fusion of two juvenile corals of different morphs will show intermediate characters between the two morphs or if it will separate into two different parts as it grows. It is also not known at what stage of development the coral develops a functional histocompatibility system.

In sponges, allograft reaction typically proceeds through an initial stage of tissue fusion or "tissue bridging" (Hildemann et al. 1980). There is a lag period before allografts display cytotoxicity in corals (Johnston et al. 1981). However, the initial stage of tissue fusion was not described in other studies on allografts of corals, nor observed in the present grafting experiment with adult colonies of *P. damicornis*. Thus it is unlikely that the fusion of primary polyps observed in this short term experiment was due to a lag period before cytotoxicity appears. However, a further study might be necessary to exclude the above possibility.

The present study shows that newly settled coral larvae derived from genotypically different colonies fuse with each other, while allografts are invariably incompatible in adult colonies.

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