Reference: *Biol. Bull.* **179**: 279–286. (December, 1990)

Ontogenetic Variation in Sponge Histocompatibility Responses

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Abstract. Grafting of adult sponge fragments (*Chalinula* sp.) led to isograft fusion and allograft nonfusion in both parabiotic and implant grafts. We conclude that adult *Chalinula* sp. individuals discriminate between self and nonself, and fuse only isogeneic fragments. In the laboratory, however, larvae and early juveniles fuse. Larvae used in the experiments were probably genetically different, even if they were asexually reproduced. These results indicate that the capacity for fusion between allogeneic individuals disappears during ontogenesis in this sponge. In some cases, multichimeras were formed when up to five larvae fused to yield a single sponge. All 37 chimeras metamorphosed and survived during 17 days of observation. Possible mechanisms for the formation of sponge chimeras during early development are discussed, as are the costs and benefits of chimera formation at juvenile *versus* adult stages. We propose that, if fusion exists in the field, it occurs between kin larvae.

Introduction

Sessile marine organisms frequently contact each other. In many instances this contact induces a recognition process during which self/nonself histocompatibility is established, resulting in acceptance or nonacceptance of the tissues involved. Intraspecific (allogeneic) encounters are frequently characterized by visible recognition events in which various responses may occur. Allogeneic histoincompatibility (nonacceptance of tissues from different conspecific individuals) has been observed in various groups of invertebrates during the past two decades: ascidians, bryozoans, stony corals, sea anemones, gorgonians, hydrozoans, and sponges (reviewed in Grosberg, 1988). The pioneering work of Wilson (1907) led to extensive research on cellular events during the reaggregation of sponge cells (reviewed in Smith, 1988; and Gramzow *et al.*, 1989), and on the consequences of sponge grafting (*e.g.*, Hildemann *et al.*, 1979; Curtis *et al.*, 1982; Smith and Hildemann, 1986).

The general view is that, even if all sponges cannot be claimed to manifest allorecognition, increasing evidence suggests that many of them are characterized by a high degree of polymorphism, and usually will not accept allografts (Smith, 1988; Van de Vyver, 1988). These results demonstrated highly diverse reactions among sponges, to both allografts (contact between individuals of the same species) and xenografts (contact between individual of different species). The reactions vary from xenograft (Paris, 1961) and allograft acceptance (formation of stable chimeras) in various sponges (Evans and Curtis, 1979; Kaye and Ortiz, 1981; Zea and Humphreys, 1985), to allograft rejection (Smith, 1988).

The genetics of sponge allorecognition, currently, is poorly known, but can be explored through allograft experiments on transitivity relationships. Transitive compatibility is defined as a situation in which individual A is compatible with B, B is compatible with C, and A is compatible with C. A non-transitive situation occurs when A is compatible with B and C, but B and C are not compatible with each other. Allograft studies of marine sponges have demonstrated transitivity (Neigel and Avise 1983, 1985; Wulff, 1986), and may imply that complete allotypic matching is required for compatibility. A difficulty in such studies among sponge populations in the field is the possible existence of clonemates derived through asexual propagation (*e.g.*, Neigel and Avise 1983; Wulff, 1986). Therefore, the tested sponges must be widely separated from each other in the field (more than the dispersal distance for an asexual propagule), to reduce the possibility of their being clonemates.

Received 11 June 1990; accepted 25 September 1990.

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The phenomenon of fusion between sponge larvae has been sporadically reported (Wilson, 1907; Burton, 1949; Warburton, 1958; Borojevic, 1967; Van de Vyver, 1970; Fry, 1971; Van de Vyver and Willenz, 1975), but has received inadequate attention. Fusion between larvae derived from the same parent might be considered as an autograft if the larvae were produced parthenogenetically, as is known in corals (Stoddart, 1983, 1984) and has been suggested for some sponges (reviewed by Fell, 1974; Bergquist, 1978; Simpson, 1984). On the other hand, if the larvae are not clonemates, then their fusion may be regarded as allograft fusion, resulting in the creation of a chimera. If adult sponges do not fuse, but their allogeneic larvae fuse, then questions of variable self/nonself historecognition responses during a sponge's lifetime, and the capacity of larvae to distinguish between self and nonself, are raised.

In the present study, we address the following questions. How do *Chalinula* sp. adults react toward isografts and allografts? How do *Chalinula* sp. larvae derived from the same parent react to each other? And what are the consequences of encounters between larvae that originated from different parents?

Materials and Methods

We studied the brooding sponge *Chalinula* sp. from the coral reefs of Eilat, Israel, on the Red Sea (29°30′N; 34°55′E), after establishing its reproduction and settlement (Ilan and Loya, 1990). Larvae were obtained by slicing adult sponges and collecting the well-developed free-swimming larvae.

Two sets of experiments on larvae were conducted. In the first set, larvae were derived from the same parent, and in the second, from different parents. In the first set of experiments, 224 larvae were obtained from 25 individual sponges. Two to ten larvae were placed in each petri dish (all derived from the same parent) to assess the possibility of fusion between two larvae (bichimera) or more (multichimera). In the second set, 3 experiments were conducted with 104 larvae taken from 14 different individuals. Every petri dish contained only two larvae, each derived from a different adult sponge. The petri dishes (bottom surface area 9.6 cm^2) were filled with 9 ml unfiltered seawater. The adult sponges used in the second set of experiments grew in the sea, 10 to 300 m apart from each other. Such distances have been considered beyond fragment dispersal in cases of frequently fragmenting sponges growing in areas affected by storms (Jokiel *et al.*, 1982; Kaye and Ortiz, 1981; Wulff, 1985). Because fragmentation and budding are not common phenomena in *Chalinula* sp., and no frequent storms occur in the study area, such a distance between the parental sponges, diminishes the possibility that these sponges could be genetically identical clonemates. The experiments in this second set were designed to introduce larvae of every sponge to larvae from each of the other sponges. The larvae for the 3 experiments in this set were obtained from 6, 4, and 4 parental sponges and had 15, 6, and 6 possible combinations of parents, respectively (25 out of the 27 possible combination were performed in duplicate). All experiments were conducted at ambient seawater temperature (25 ± 1°C).

The tendency of larvae to aggregate was tested in the second set, using the statistical analysis of the goodness of fit (Sokal and Rohlf, 1969). According to our definition, aggregation occurs when two larvae establish and remain in contact. To calculate this occurrence, a hypothetical *Chalinula* sp. larva was considered to be a rectangle of 1 × 0.5 mm (0.5 mm^2). These two figures are larger than those of any *Chalinula* sp. larva measured (Ilan and Loya, 1990) and are therefore considered to be conservative. In a petri dish of 960 mm^2 bottom area, there are 1920 rectangles of 0.5 mm^2. The second larva will contact the first one only if it settles on top of the first larva, or in one of its four neighboring rectangles. Given a random larval settlement in a dish, the probability of two larvae contacting is: $5 \times (1/1920)$. Because 52 pairs of larvae were used in these experiments, there would be random contact between the larvae in $52 \times 5 \times (1/1920) = 0.135$ of the pairs. Any significantly higher value than this prediction, implies larval aggregation.

Two grafting experiments between fragments of adult sponges, involving two different protocols, were conducted *in situ* in front of the Marine Biological Laboratory, Eilat, at least 1 m below lowest tide. *Chalinula* sp. fragments of about 3 × 4 cm were attached to each other and to a fiberglass net anchored to the bottom. Fragments were taken from sponges situated 10 to 300 m apart from each other on the coral reef. We used five sponges in each of the two experiments, with all cross combinations (with duplicates) of allogeneic interaction made. To determine whether this species is capable of fragment fusion, all the experiment sponges were also isogeneically grafted. In the first experiment, intact external surfaces of sponges (pinacoderms) were placed in contact (parabiotic grafts). Neigel and Avise (1985) considered this technique to be more reliable than implant grafts in which a block of donor's tissue is implanted into a recipient. However, following the suggestion of Johnston and Hildemann (1982) that a reaction may be very slow, and a review by Smith (1988) on the involvement of mesohyle (inner nonfiltering) cells in the process of acceptance or rejection of grafts, we set up a second experiment in which contacts were made between fragments of mesohyle to speed the reaction process. We used equal sized fragments, and not the implant technique, to avoid a possible effect of recipient size on

the donor's block (Hildemann *et al.*, 1980). The grafting experiments were observed for three months.

Samples of grafted zones on the sponges were fixed in 2.5% glutaraldehyde buffered in seawater for scanning electron microscopy, then washed, dehydrated in graded ethanol series, critical-point-dried, coated with gold-palladium and viewed in a JEOL JSM-840A SEM.

Results

When free-swimming *Chalinula* sp. larvae (n = 224), derived from the same parent sponges, were put together in a dish, 44% fused (Table I). In dishes with more than two larvae, there were cases of fusion between two larvae (bichimera as in Fig. 1a); 3 to 5 larvae (multichimera) also fused, metamorphosed successfully, and gave rise to single sponges (Fig. 1b). In all, 37 chimeras were observed (Table I). *Chalinula* sp. larvae fused during different stages: as free swimming larvae (Fig. 1a, 1b), or as post larvae shortly after attachment and metamorphosis, when they grew toward each other and fused at the contact zone (Fig. 1c). In some cases, free-swimming larvae settled on, and fused with, recently metamorphosed sponges.

In the second set of experiments, each of the pair of larvae in a dish was taken from a different sponge. In 19 pairs out of 52 (36.5%), the larvae fused. The observed number of fused pairs is significantly higher than the expected (0.135) from random settlement ($P \ll 0.001$, goodness of fit test). The larvae settled in all areas of the dishes and were not confined to certain microhabitats (*e.g.*, corners, center); therefore the aggregation was not due to external pressures. In two cases, chimeras redivided into two distinct individuals, after one to three days, although the duplicate of one of these pairs, which also produced a chimera, did not separate. Larvae and chimeras remained alive during the 17 days of observation in the first larval experiment, and in the second, they remained alive for 39 days of observation.

Isografts conducted between fragments of adult *Chalinula* sp., fused within three to ten days, whether the contact zone was between exopinacoderms (parabiotic grafts) or between mesohyles (Fig. 2). Fusion was characterized by a continuum of the choanosome, with no apparent boundary at the grafted zone. These fragments remained fused over 3 months of observation. When allografting was performed between fragments taken from the same sponges that had been used for the isografting, no fusion was observed between the 20 allografts (Fig. 3). Scanning electron micrographs of allografts attached at the internal (choanosomal) zone of the fragments, revealed that within 3 d, a gap of about 100 μm was formed between the fragments, with spicules erected toward this zone (Fig. 3b, 3c). Each fragment developed a pinacoderm at the grafted area, with a separation between them (Fig. 3d). No aggressive interactions were observed between the nonfusing fragments in the allografts. When parabiotic grafts were employed (20 pairs), both fragments remained intact, but fusion did not occur, nor was any rejection phenomenon observed over the three months of the experiment.

Discussion

The existence of self/nonself recognition among adult *Chalinula* sp. is strongly indicated in this study. Fusion between all fragments involved in isografts occurred, regardless of the grafting method used (parabiotic *versus* implant grafts), establishing that members of this species can fuse isogeneically. However, when grafts were made between allogeneic fragments of the same individuals used in isografting, fusion did not occur in any of the paired fragments.

Chalinula sp. larvae have a statistically significant tendency to aggregate. Molecules termed aggregating factors occur in some sponges (Moscona, 1968), and such molecules are known to facilitate species-specific and non-

Table I

Occurrence of fusion between larvae taken from the same Chalinula *sp. individual*

# Larvae in dish	# Dishes	% Dishes with fusion	% Fused larvae	# Chimeras of			
				2	3	4	5 Larvae
2	8	37.5	37.5	3			
3	4	50.0	41.7	1	1		
4	27	55.6	30.6	12	3	0	
5	4	75.0	65.0	0	0	2	1
6	3	33.3	11.1	1	0	0	0
7	3	100.0	71.4	2	1	2	0
9	1	100.0	66.7	0	2	0	0
10	2	100.0	95.0	0	5	1	0
Total	52	57.8	44.2	19	12	5	1

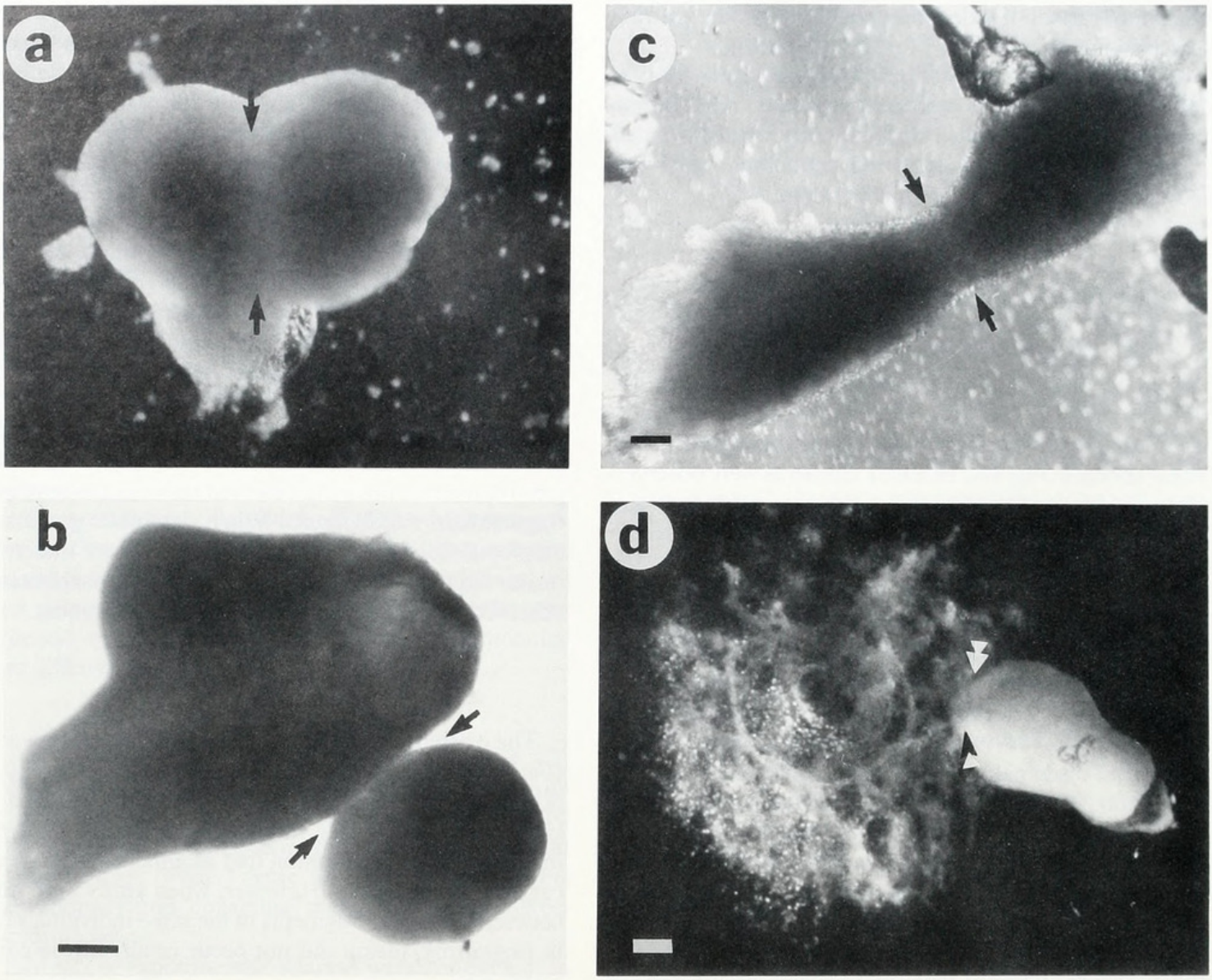

Figure 1. *Chalinula* sp. larval and post-larval fusion. (a) A pair of fused larvae, 2 h after initial contact. (b) A pair of fused larvae, 24 h after fusion, start to fuse with a third larva. (c) Fusion of two post-larvae. Fusion followed their settlement in proximity. (d) A new larva starts to settle on and fuse with a two-day old post-larva. In all the light micrographs, arrows indicate the contact zone (scale bar = 200 μm).

specific reaggregation of dissociated sponge cells (reviewed by Muller, 1982; Coombe and Parish, 1988).

The fusion of larvae derived from the same parent may have occurred for several reasons: (1) the larvae may lack a capacity for self/nonself discrimination; (2) they may possess such discrimination, but may also express an inhibition of the rejection mechanism; and (3) the larvae may have been genetically identical (products of parthenogenetic reproduction), thus resulting in the fusion of grafts that were actually isografts and not allografts.

The last cause of larval fusion is less likely, because the larvae were taken from sponges 10 to 300 m apart from each other in their natural habitat; therefore they were probably genetically different. Thus, the larvae that fused in the experiments were probably genetically different, even if asexual development of larvae occurs in *Chalinula* sp., which is unlikely (Ilan and Loya, 1990). These results differ from the situation reported by Van de Vyver and Willenz (1975), who studied the freshwater sponge *Ephydatia fluviatilis* and described larval fusion as occurring only between larvae belonging to the same strain.

If indeed larvae were incapable of self/nonself discrimination, the results with adult grafting indicate acquisition of this capability during ontogenesis. Juvenile immunological incompetence is well known among vertebrates (Cooper, 1976) and has been suggested also for corals (*e.g.*, Duerden, 1902; Lang, 1971; Hidaka, 1985) and hydroids (Teissier, 1929; Schijfsma, 1939). The tendency of *Chal-*

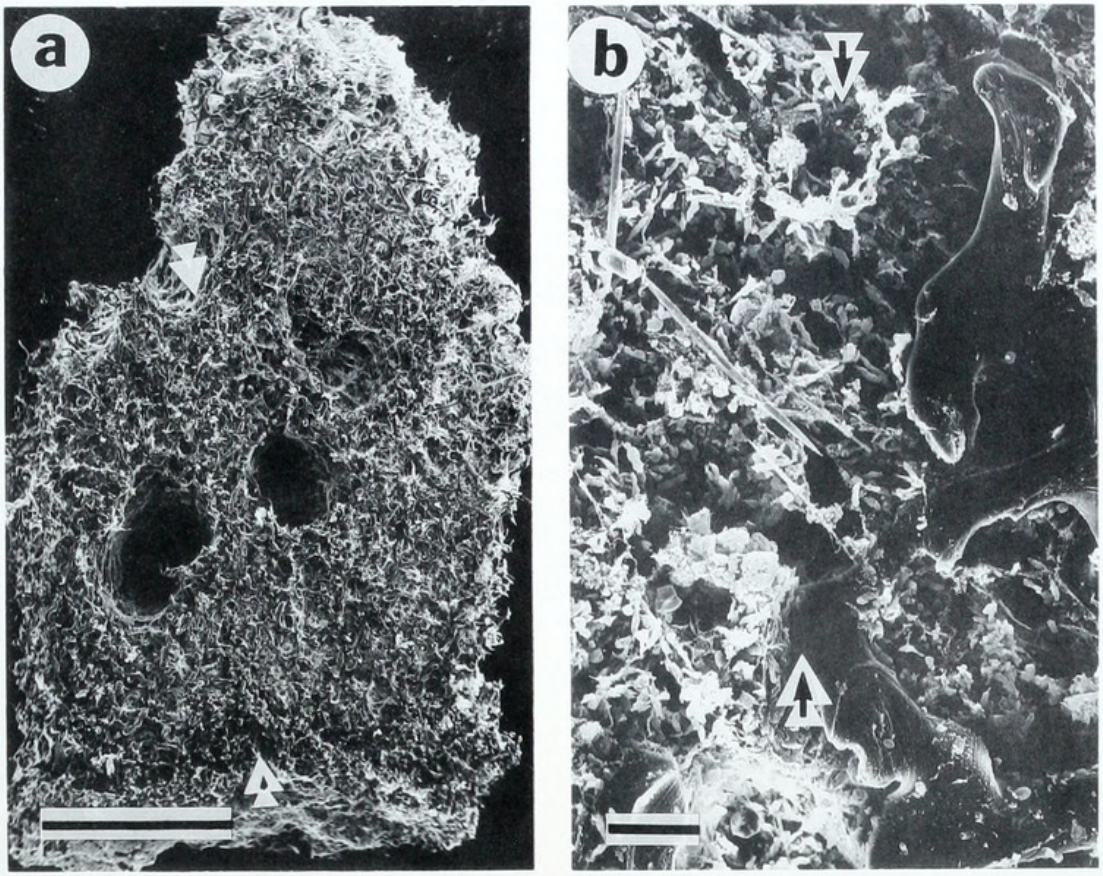

Figure 2. *Chalinula* sp. adult isograft viewed through scanning electron microscope. (a) Two complete grafted fragments, with arrows indicating toward the fusion at the contact zone (scale bar = 1 mm). (b) Higher magnification of the contact zone shows a continuum of cells (scale bar = 100 μm).

inula sp. larvae to form intraspecific aggregates demonstrates, however, some recognition capacity (though only on the species level). Lack of reaction against nonself might have been due to a lag period after which a rejection, separation, or resorption of one partner in the chimera by the other could have occurred, as is known for tunicates (Scofield *et al.*, 1982; Rinkevich and Weissman, 1987, 1989). The present study indicates that if any lag period exists, it must be at least 17–39 days long. However, a process that cannot be excluded is cell lineage competition (Buss, 1982), in which cells with different genotypes within one body may compete for position in the germ line. Finally, another option is that, although capable of differentiating self from nonself, larvae were unable to inhibit fusion due to lack or inactivation of a rejection mechanism, a situation analogous to self-tolerance in vertebrate T or B-cells (*e.g.*, Basten, 1989; Nossal, 1989; Schwartz, 1989).

Conspecific larval aggregation by *Chalinula* sp., followed by fusion with no rejection raises the question: what are the benefits of creating chimeras during larval or early post-larval stages, in contrast to the possible disadvantages (reflected by allograft incompatibility) of having chimeras at the adult stage? In the juvenile stage, the most important advantage may be the chimera size, which is larger than any of the individuals that created it. Small body size in marine invertebrates is often accompanied by high mortality, whereas larger size results in higher survivorship (*e.g.*, Loya, 1976; Ayling, 1980; Hughes and Connell, 1987). Hence, individuals that fuse, forming a chimera, immediately increase their total size and probably also their survivorship. Another possible benefit suggested for chimeras is early reproduction, because sexual maturity is also often size-related (reviewed in Harvell and Grosberg, 1988). Thus, reducing generation length may yield an increasing number of offspring per unit time, compared with a similar genotype having a longer generation time. Buss (1982) argued that chimeras might be advantageous if there is mixing of cells from all partner genotypes. Chimeras, being larger are more likely to suffer partial- rather than whole-colony mortality, with surviving cells bearing all genotypes. Finally, having a compound genotype, a chimera may gain more physiological resistance to different environmental conditions than any of its members separately (Buss, 1982; Grosberg and Quinn, 1986).

Most of the proposed benefits (except for physiological resistance) are consequences of larger body size of a chimera *versus* its members. Therefore, adults, which have already reached a substantial size, do not need to fuse with others to raise their survivorship or to reduce the

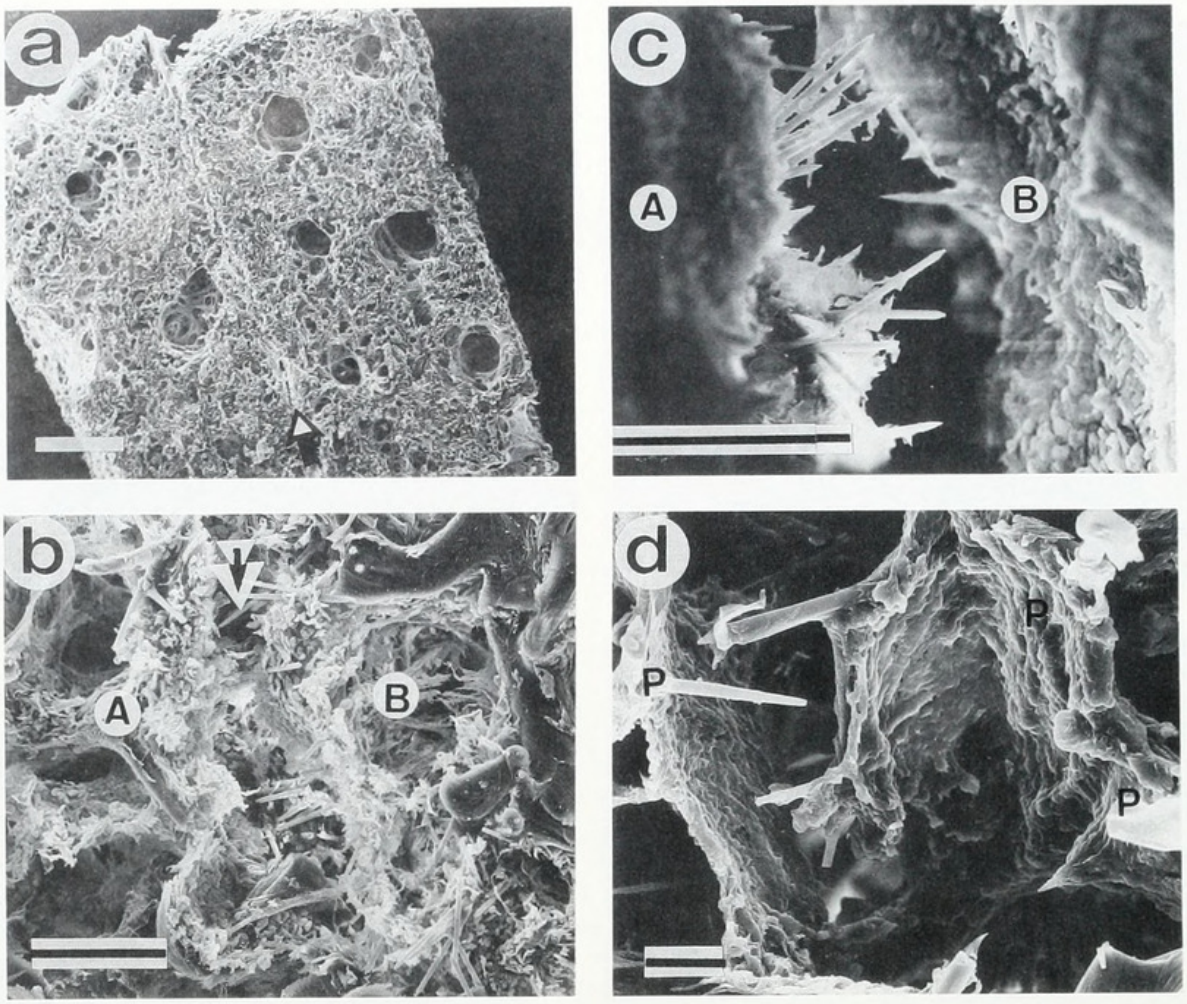

Figure 3. *Chalinula* sp. adult allografts, made by placing together the mesohyl of two fragments from different individuals and viewed through a scanning electron microscope. (a) Two allogeneic fragments. Arrow indicates the grafted area (scale bar = 1 mm). (b) Fragments A and B with a gap at the contact zone (scale bar = 100 μm). (c) Higher magnification, reveals spicules erected toward the contact zone, presumably due to cell disappearance from this area (scale bar = 100 μm). (d) Formation of a pinacoderm (P) layer by each fragment at the contact zone (scale bar = 30 μm).

time to onset of reproduction, which they have already started. We suggest, therefore, that the nonfusion of adult *Chalinula* sp. evolved because the disadvantages and risks involved are not outweighed by the chimeric benefits of larger total size.

Considering the costs of participating in a chimera, we first assume that an organism acts to maintain its integrity, in order to pass on its genotype to the next generation. Several potential deleterious consequences of creating chimeras were proposed in the literature. Buss (1982, 1983) suggested a possible parasitism by one member of a chimera on the other; by differentiating its germ cells to gametes, it would take advantage of the other member's investment in somatic tissues for maintenance. Other workers have demonstrated oriented translocation of material in coral chimeras (Rinkevich and Loya, 1983), possible transmission of pathogens (Buss, 1982), or in an ascidian, total resorption of one member's soma by its partner, under laboratory mariculture (Rinkevich and Weissman, 1987, 1989). In this study, although all the chimeras survived at least 17 days, the fate of the cells of each partner was not determined.

Chalinula sp. larval fusion has been observed experimentally in this study in the laboratory. However, its frequency in nature is unknown. Theoretically, the chances of contact between *Chalinula* sp. larvae from different sources in the field are small. Its year-round reproductive pattern (Ilan and Loya, 1990) leads to a small number of free-swimming larvae in the population at any given time. This fact, together with the large distance between adult colonies, relative to larval size, plus rapid larval settlement (1 to 8 h after release) (Ilan and Loya, 1990), contributes to the low probability of larval contact. Nonetheless, larvae brooded in the same sponge, even if they are genetically different (produced sexually), may overcome most of the barriers to larval fusion in the field. Such larvae are in close proximity and, if spawned synchronously, may settle together and

fuse. Fusion between kin larvae may provide an additional selective advantage. Kin larvae partially share genotypes, therefore the survival of each is a partial success for the other genotype. Thus, if larval and newly post-larval chimeras of *Chalinula* sp. do occur in the field, we assume they will be primarily among kin.

Acknowledgments

We are grateful to B. Rinkevich for many valuable discussions. We thank R. Seggev Ben-Hillel who helped in maintaining the larvae, and Z. Goldberg for assistance in the field work. A. Colorni and A. Shoob took the light microscope photographs. Y. Delarea and F. Skandrani helped with the electron microscope. We appreciate the time and effort spent by T. P. Hughes and B. Rinkevich and two anonymous reviewers in critically reading earlier versions of the article. This work was partially supported by a grant from the Ben-Gurion fund for encouragement of research—Histadrut—the general federation of labor in Israel.

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