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Nontransitive patterns of historecognition phenomena in the Red Sea hydrocoral *Millepora dichotoma*

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Abstract. Allogeneic assays were conducted in situ on the Red Sea hydrocoral *Millepora dichotoma*. Forty-five pairwise combinations among ten colonies and ten control isografts were set up in four replicates each (180 and 40 pairs, respectively) and followed for up to 8 mo in the Gulf of Eilat, Red Sea in 1992–1993. In 42 allogeneic combinations we recorded a reproducible and unilateral tissue and skeleton overgrowth which developed within the first 10 wk up to 20 mm. Following the development of these primary overgrowths, four types of secondary responses were observed among most incompatible combinations: reversals in overgrowth directionality, tissue necroses, stand-offs and abnormal growth patterns. Secondary responses within a given set of replicates of most allogeneic combinations were characterized by high variability in type and intensity of response. Based on the outcomes of primary overgrowths, a complex nontransitive hierarchy was constructed for the ten colonies. All isografts and three allogeneic combinations fused within 3 wk. Fusion pattern between the three allogeneic combinations was nontransitive: one *M. dichotoma* colony repeatedly fused with two other colonies (four assays each). However, these two colonies not only did not fuse with each other, but one of them repeatedly overgrew its confrére. In the third compatible combination, the most superior and the most inferior colonies in the network of hierarchies among the ten colonies, fused in all tested assays. These results are compared with allogeneic outcomes in other cnidarians, where nontransitive fusion between allogeneic colonies have never been recorded.

Introduction

The recognition of self vs non-self in lower invertebrates is a well documented phenomenon. In all studied taxa, isografts are always compatible, while allografts and xenografts are rarely accepted (Cooper et al. 1992). On the other hand, the genetic rules governing invertebrate immunity were elucidated for only a limited number of taxa (protochordates: Oka and Watanabe 1960, Sabbadin 1962, Scofield et al. 1982; ciliates: Beale 1990).

Cnidarians, being one of the most ancestral invertebrate groups, have attracted the interest of comparative immunologists, who used them as model systems for studying the evolution of vertebrate immunity (Cooper et al. 1992). Most of the studies on cnidarian immune responses were performed in the field using anthozoans such as reef corals and sea anemones as experimental organisms (Leddy and Green 1991). These studies usually followed allogeneic interactions on the morphological level, characterizing the types and expressions of responses, variety of mechanisms, immunological memory and maturation, etc. (Hildemann et al. 1977, Bak and Criens 1982, Rinkevich and Loya 1983a, Hidaka 1985, Lasker and Coffroth 1985, Chornesky 1991, Salter-Cid and Bigger 1991).

In the Hydrozoa, most of the studies on allogeneic and xenogeneic interactions were performed on the genera *Hydra* and *Hydractinia*. (e.g. Hauenschild 1954, 1956, Toth 1967, Znidaric 1981, Buss et al. 1984, 1985, Bosch und David 1986, Shimizu and Sawada 1987, Lange et al. 1989), revealing several types of antagonistic responses. In allogeneic encounters, formation of hyperplastic stolons (Ivker 1972) or nematocyst accumulation and discharging was recorded (Buss et al. 1984, Lange et al. 1989). In xenogeneic encounters, epithelial cells, which recognized and phagocytized the foreign cells, were observed (Bosch and David 1986).

The hydrocoral, *Millepora* spp. possesses a self-nonself discrimination system as do other hydrozoans (Boschma 1948, Müller et al. 1983). In some allogeneic encounters, it has been suggested that nonself recognition is restricted only to those areas with a high polyp density (Müller et al. 1983). In xenogeneic encounters, members of the genus *Millepora* overgrew several Caribbean gorgonians (Wahle 1980) and scleractinians (Chornesky 1991) but were overgrown in the Red Sea by a sponge, a scleractinian coral and by an alcyonarian coral (Rinkevich et al. 1993). All past studies on *Millepora* spp.

immune responses were, however, based on short term observations of naturally occurring allogeneic and xenogeneic encounters. A detailed follow-up study on allogeneic responses between *Millepora* spp. colonies, as was conducted on other cnidarians (Hildemann et al. 1977, Rinkevich and Loya 1983a, Salter-Cid and Bigger 1991, Rinkevich et al. 1994, Chadwick-Furman and Rinkevich in press), has not yet been carried out. Here, we followed in situ, allogeneic interactions among *M. dichotoma* colonies for up to 8 mo. Replicate assays of all 45 possible pairwise combinations among ten *M. dichotoma* colonies resulted in most of the cases (42) in the expression of a variety of incompatible responses. In three combinations, fusion between allogeneic tissues was recorded.

Materials and methods

Study organism

While the taxonomy of the genus *Millepora* is still a subject of extensive studies (Boschma 1949, de Weerdt 1981), this genus emerges as an important framework builder of many Atlantic and Indo-Pacific coral reefs (Lewis 1989).

Millepora dichotoma Forskål is a common inhabitant of shallow water coral reefs in the Gulf of Eilat, where it is usually found at 0 to 5 m depth (the *Millepora* zone; Loya 1972). This species is characterized by a fast growth rate and rapid regeneration of tissue lesions (Fishelson 1973). It occurs in a variety of growth forms including branching, massive, encrusting and intermediate forms (Vago 1989). The habitus of any *M. dichotoma* colony is probably affected by a variety of environmental and biological factors (Vago 1989), as was also claimed for some Caribbean *Millepora* species (de Weerdt 1981).

Field experiments

The present study was carried out on the coral reef adjacent to the H. Steinitz Marine Biology Laboratory at Eilat, Red Sea, Israel, at a depth of 5 to 7 m using SCUBA.

Millepora dichotoma colonies of only the branching morph were sampled, since this form may easily be subcloned in situ to many similar-sized colony fragments (subclones), causing minimal damage to both the colonies and the fragments. Branches, 7 to 14 cm long, were carefully detached from ten large and healthy colonies (labeled A to J) using side cutting pliers. They were secured to submerged concrete tiles by preglued plastic clothespins (Rinkevich and Loya 1983a). All 45 possible pairwise allogeneic combinations and ten control isografts (within colony grafts) were set up in four replicates each. Isolated subclones were allowed to acclimatize on the tiles to the experimental conditions for up to 2 wk. Thereafter, branches were positioned in pairs, so that the tips of each pair-branches contacted each other gently, without injuring their tissues. A total of 180 allogeneic and 40 isogeneic pair-branches were observed every 2 to 3 wk and photographed (using a Nikonos V camera equipped with a 35-mm lense and a close-up attachment) every 6 to 8 wk, from May 1992 until January 1993.

Results

Allogeneic encounters among *Millepora dichotoma* conspecifics were characterized by either compatible (tissue fusion; Fig. 1a) or by incompatible (Fig. 1b–h) responses.

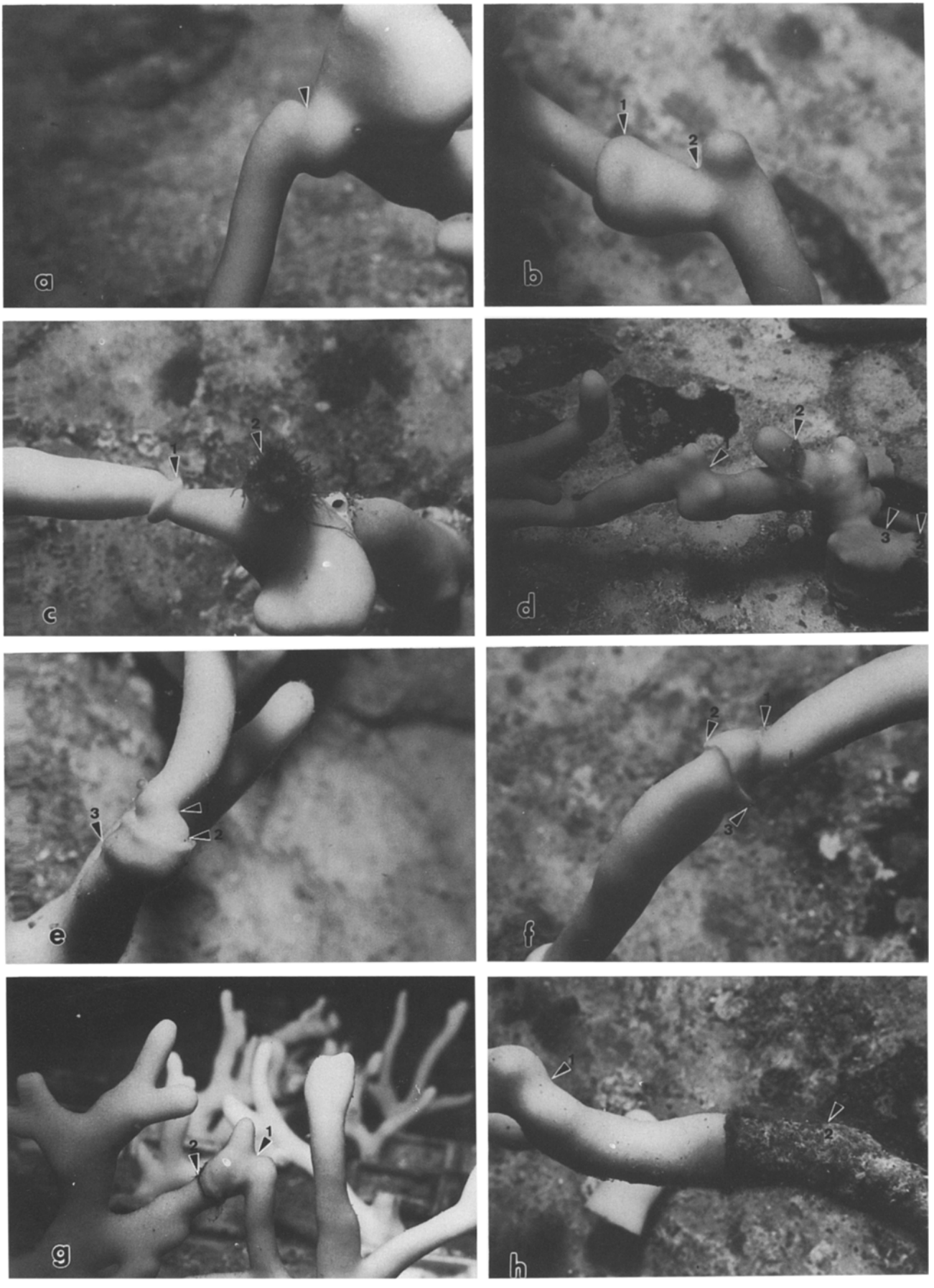
Incompatible responses

Out of total 180 allogeneic assays, 168 (of 42 allogeneic combinations) resulted in incompatible responses (Table 1). All replicate assays of 41 combinations started with unilateral overgrowth, where one partner in each assay overgrew its confrére (Fig. 1b). Tissue overgrowth was first observed in situ from 2 wk (colony-combination BJ) to 10 wk (combination IJ) after tissue-to-tissue contacts. In one combination (EI), none of the four replicates developed overgrowth responses. Instead, a suture (sensu Chadwick-Furman and Rinkevich in press), at the border line between both partners, was formed following 10 wk of interaction (Fig. 1c).

In 33 incompatible pairwise combinations, all replicates consistantly resulted in the same directionality for tissue overgrowths. In five combinations, one out of the four replicates in each combination overgrew its confrére in a direction opposite to the other three. In three combinations (FG, FA, IJ), both partners overgrew each other in an equal number of replicates (Fig. 2). Starting time for overgrowth was highly variable within most combinations. For example, overgrowth was first observed in the four replicates of combination CH after 2, 4, 6 and 10 wk respectively, following contacts. In all cases of overgrowths, a visible borderline clearly demarcated both allogeneic tissues, which were structurally "cemented" (Fig. 1b–h). This borderline was the site of separation when interacting pairs were decalcified (formic acid and sodium citrate). When mechanical force was applied, the original area of contact (i.e., before the onset of overgrowth) was always the breaking-up site. All above overgrowths will be termed here as "primary allogeneic responses".

Based on the results of primary overgrowth vectoriality, a transitive and nontransitive hierarchical network of overgrowths among the ten colonies was established (Fig. 2). Colonies J and I emerged as the most superior in

Fig. 1. *Millepora dichotoma.* Alloimmune interactions between *M. dichotoma* conspecifics. (a) Colony combination AC. Fusion, as recorded after 16 wk from tissue contact, characterized by continuous tissue and skeleton across contact area. Arrow indicates site of original contact. (b) Combination AB. Primary overgrowth of one branch over the other. Arrows indicate: (1) thickened zone formed by the overgrowing tissue; and (2) site of original contact. (c) Combination EI. A suture at the borderline between two branches exhibiting no overgrowth response, 12 wk from contact. Arrows indicate: (1) suture; and (2) a necroting area on which algae were settled. (d) Combination CJ. Overgrowing tissue advanced up to 20 mm on colony C. Arrows indicate: (1) original contact zone; (2) necrosis; and (3) the overgrown clothespin which holds the branch. (e) Combination AI. Reversal in overgrowth directionality. In the primary response, the right branch overgrew the left one, and left the branch overgrew the right one after reversal. Arrows indicate: (1) site of original contact; (2) present borderline between the tissues; and (3) reversal area in the shape of a ring. (f) Combination GJ. Simultaneous overgrowth of two branches. Arrows indicate: (1) site of original border; (2) the right branch overgrowing the left partner; and (3) vice versa. (g) Combination CF. A necrotic line on the borderline between two allogeneic tissues. Arrows indicate: (1) the original contact zone; and (2) the necrotic line. (h) Combination AH. Death of an overgrown branch. Arrows indicate: (1) the site of original contact; and (2) the dead branch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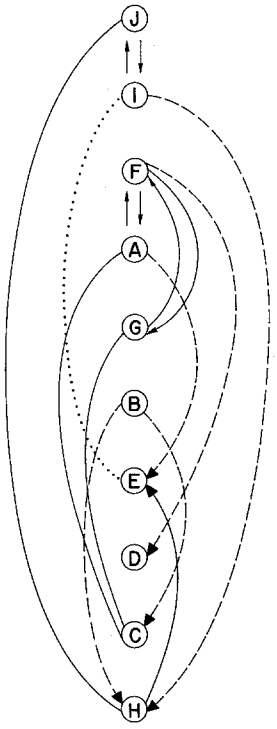

Fig. 2. *Millepora dichotoma.* A hierarchical network of overgrowths among ten allogeneic *M. dichotoma* colonies (A–J). Colonies positioned higher in the scheme, repeatedly overgrew all lower positioned colonies in all replicates. Lines without arrowheads show fusion responses; dotted line indicates neither overgrowth nor cytotoxic response; dashed lines with arrowheads indicate directionality of overgrowth in three replicates (while one replicate responded in an opposite direction); lines with large arrowheads indicate overgrowth of the dominant colony by an inferior one (in all replicates); double arrows with small heads indicate reciprocal overgrowth by an equal number of replicates

this network: they both overgrew seven other colonies and reciprocally overgrew (in two replicates) and were overgrown by each other (two replicates). Colony I did not exhibit overgrowth or cytotoxicity at all with E, and colony J fused (see below) with colony H, which appeared to be the most inferior one. This colony was overgrown by seven colonies, overgrew only colony E and fused with the superior colony J. A circular hierarchy was established among the four inferior colonies E, D, C and H: colony E overgrew D and C but was overgrown by the most inferior colony H. The rest of the hierarchies were fully transitive or showed some reciprocal overgrowths and fusions (Fig. 2).

These primary allogeneic responses (Figs. 1, 2; Table 1) were established and developed within the first 10 wk (maximal time recorded in combination IJ) following tissue contact. During this period, overgrowing tissue advanced up to 20 mm over the confrére's skeleton (in combination CJ; Fig. 1 d). After the establishment of the primary allogeneic responses (unilateral overgrowth), we documented four other types of responses, termed here as "secondary responses". These secondary responses included reversals in overgrowth directionality (sensu Chornesky 1989, Chadwick-Furman and Rinkevich in press), tissue necroses, stand-offs and abnormal growth patterns.

Reversals in overgrowth directionality occurred within 17 combinations, where at least one replicate in each combination exhibited this phenomenon. However, in only two combinations (AI and BG) did all replicates develop overgrowth reversals. In some of the cases (such

Table 1. *Millepora dichotoma.* Allogeneic responses of 45 pairwise combinations done between ten *M. dichotoma* colonies (A–J) as recorded after 9, 23 and 33 wk. < or >: overgrowth directionality; N: necrosis; so: stand off; x: dead individual branchess. Number of pair-replicates given

Combination	Time		
	After 9 wk	After 23 wk	After 33 wk
AB	2A>B; 2so	3A>B; 1A<B	3A>B; 1N on B
AC	4 fusion	4 fusion	4 fusion
AD	3A>D; 1so	2A>D; 2so	1A<D; 3so
AE	3A>E; 1so	2A>E; 2N on E; 2so	1A>E; 2A<E; 1N on A
AF	2A>F; 2N on A	2A>F; 1A<F; 1so	1A<F; 2N on F; 2so
AG	2A>G; 2so	4A>G	4A>G; 1Ex
AH	4A>H	4A>H; 1Hx	4A>H; 1Hx
AI	4A<I	IA>I; 3A<I	4A<I
AJ	4A<J	4A<J; 2Ax	2A<J; 1N on A; 4Ax; 1Jx
BC	1B>C; 2B<C; 1so	3B<C; 1so	4B<C
BD	2B>D; 2so	3B<D; 1B>D	2B>D; 2B<D
BE	3B>E; 1so	3B>E; 1B<E; 1N on B	3B>E; 1B<E
BF	2B<F; 2so	2B>F; 2B<F; 1N on B	4B>F
BG	4B<G	3B<G; 1so	3B>G; 1B<G
BH	3B>H; 1so	2B>H; 2N on H; 1B<H	2B>H; 1B<H; 1Hx
BI	4B<I	4B<I	4B<I
BJ	2B<J; 2Bx; 2Jx	1B<J; 4Bx; 2Jx	1B<J; 4Bx; 3Jx
CD	1C<D; 3so	4so	1so; 3Cx; 3Dx
CE	1C<E; 3so	3C<E; 1C>E 1N on C	2C>E; 1C<E; 1so; 2Ex
CF	4C<F	4C<F; 2N on C	1C<F; 1so; 2Cx; 2Fx
CG	4 fusion	4 fusion	4 fusion
CH	1C>H; 3so	3C>H; 1N on H	4Cx; 4Hx
CI	4C<I	4C<I	4C<I; 2N on C
CJ	2C<J; 2so	1C<J; 3so	2C>J; 2Cx; 4Jx
DE	4so	4so	1D>E; 3so
DF	2D<F; 2so	3D>F; 1D<F; 2Fx; 1so	2D<F; 2so
DG	4D<G	4D<G	4D<G
DH	4D>H	4D>H; 1N on H	2D>H; 1N on H; 1so
DI	4D<I	4D<I	3D<I; 1D>I
DJ	3D<J; 1N on D	3D<J; 1so	1D<J; 2N on J; 2so
EF	4so	1E>F; 1N on F; 2so	2E>F; 2E<F
EG	3E<G; 1so	2E>G; 1E<G; 1N on E	3E<G; 1E>G
EH	4so	4E<H	2E>H; 1E<H; 2so
EI	4 no response	4 suture and so	4 suture and so
EJ	4E<J	4E<1	1E>J; 2E<J; 2so; 1Ex; 1Jx
FG	2F>G; 2F<G	2F>G; 1F<G; 1so	1F>G; 1F<G; 2so
FH	4F>H	4F>H; 2N on H	2F>H; 1Fx; 1Hx
FI	4F<I; 1N on F	3F<I; 1N on F	1F>I; 3F<I; 2Fx
FJ	2F<J; 1N on F; 2so	1F<J; 2so; 1Fx	1SO; 2Fx; 1Jx
GH	4G>H	4G>H	4G>H; 1Hx
GI	4G<I	4G<I	3G<I; 1G>J
GJ	2G<J; 2so	2G<J; 2N on G	1G>J; 2G<J; 2so
HI	2H>I; 2H<I	1H<I; 3so	1H>I; 3so; 1Hx
HJ	4 fusion	4 fusion	4 fusion
IJ	4so	4so	2I>J; 1I<J; 1so

as EG), the directionality of overgrowth changed twice during the course of the study (8 mo). Reversals were usually characterized by the formation of a calcareous "ring" at the most distant point that was covered during primary overgrowth by the advancing tissue of the overgrowing branch (Fig. 1e). Several pair-branches overgrew each other simultaneously at different points along contact areas (Fig. 1f; see also Chornesky 1991). Tissue necroses were observed from time to time, in most cases at the border zone of interacting branches. Pair-combinations BD and DI did not develop any necroses at all during the 8-mo study; in others, necrotic lines (Fig. 1g), points (Fig. 1c, d) or whole branch death were evident (Fig. 1h). Dead branches were settled by algae and other sedentary organisms and sometimes by the "superior" colony's tissue. Combination-specific cytotoxicity (hierarchical) was not documented here among any of the incompatible combinations. In several combinations, a stand-off (sensu Chornesky 1989) occurred irregularly after the establishment of primary response. Abnormal growth as a response to allogeneic challenges was characterized by thickening of the overgrowing branches at the interaction areas (Fig. 1b).

Secondary responses were not consistent within different pair-branches of a given colony combination, neither in the directionality nor in the types of response. With time, the variability of outcomes among pair-branches increased. In Table 1 we present this pattern of responses as a function of time. For example, combination DH: after 9 wk of interaction, D overgrew H in all four replicates. After 23 wk one necrotic spot was observed on one branch of H (D still overgrew H in all replicates), and after 33 wk, D overgrew H in only two assays, was in a stand-off position within one, and tissue necrosis on the branch of H without overgrowth was evident on the fourth. The general state of health of incompatible pairs declined over time, and necrotic areas or whole branch death became more frequent the longer the experiment ran. None of above responses were ever observed in compatible (fusing) pairs.

Compatible responses

All 40 pairs of the isografts and 12 pairs of three allogeneic combinations (AC, CG and HJ) fused within 2 to 3 wk following contact and remained fused until termination of the experiment, 8 mo later (Fig. 1a). We set up additional four pairs of each of the three compatible allogeneic combinations. Again, all these pairs resulted in complete fusion (data not shown). About 5 to 6 mo after initial tissue-to-tissue contacts, the site of original border between fused branches could no longer be distinguished. Furthermore, fusion areas were no longer sites of breakage when mechanical force was applied, as opposed to incompatible pairs. Decalcification of skeletons (after tissue fixation) did not separate between the tissues of compatible branches, as occurred in incompatible ones. Compatible pairs appeared healthy during the whole course of the study and showed a very low mortality. Mortality occurred in only one replicate of combination HJ, where tissue necrosis had started, however, away from the site of tissues contact, at the attachment point of branch J to the holding clip.

Discussion

Incompatible allogeneic responses

We have documented here a nontransitive scheme of allogeneic overgrowth hierarchies among incompatible *Millepora dichotoma* colonies, similar to the allogeneic hierarchical patterns of the scleractinian corals *Stylophora pistillata* (Chadwick-Furman and Rinkevich in press) and *Acropora hemprichi* (Rinkevich et al. 1994). In the only other hydrozoan (*Hydractinia echinata*) for which a hierarchy of allogeneic interactions was established, a transitive pattern of overgrowths was revealed (Ivker 1972). It has been suggested that this transitive hierarchy is correlated with the relative growth rate of interacting colonies. Fast growing *H. echinata* colonies were located higher in this hierarchy than relatively slow growing ones. Such correlation has not been tested in *S. pistillata* (Chadwick-Furman and Rinkevich in press), *A. hemprichi* (Rinkevich et al. 1994) or *M. dichotoma* (present study). However, the nontransitive pattern of hierarchies found in these corals suggests that other mechanisms may shape the outcomes of interactions in addition to the supposed relative growth rates.

The nontransitive hierarchies reported for allogeneic interactions in *Stylophora pistillata* (Chadwick-Furman and Rinkevich in press) and *Acropora hemprichi* (Rinkevich et al. 1993) differ in two main characteristics from the primary overgrowth in *Millepora dichotoma*. (1) Time-scale: alloimmune responses were stabilized in *S. pistillata* after more than 1 yr, and in *A. hemprichi* after up to 7 mo. In *M. dichotoma*, in contrast, short-term bioassays of 3 to 10 wk were enough to reach a primary alloimmune responses. (2) Repeatability: all assays done on *S. pistillata* (Rinkevich and Loya 1983a, Chadwick-Furman and Rinkevich in press) and the vast majority of those done on *A. hemprichi* (Rinkevich et al. 1994) consistently revealed a one-way pattern of overgrowth directionality. In *M. dichotoma* only 78% of incompatible allogeneic combinations showed this pattern for primary responses.

The secondary allogeneic responses were much more variable, and a pattern in the type, vectoriality, duration or severity of response could not be established. This resembles the results reported for xenogeneic antagonistic responses between competing coral species (Bak et al. 1982, Logan 1984). It is possible that this characteristic of secondary allogeneic responses in *Millepora dichtoma* resulted from other biological and environmental parameters (cf. Bak et al. 1982) rather than from effector mechanisms directly correlated with the process of nonself recognition. This suggestion is also supported by the observed declining condition (state of health) of interacting branches over time, which is followed by the increased number and size of tissue death (Table 1). During the development of primary responses, all interacting

branches were in good condition; therefore, primary allogeneic responses may be regarded as genuine characteristics of nonself recognition phenomena.

Compatible responses

In the cnidarians, recontacting separated clonemates result in tissue fusion. This type of fusion is characterized by the formation of continuous tissue and skeleton, where applicable, across the contact area. Fusion between allogeneic tissues in various cnidarian taxa has been reported by several authors (e.g. Hauenschild 1954, 1956, Buss et al. 1985, Heyward and Stoddart 1985, Resing and Ayre 1985, Willis and Ayre 1985, Yamazato and Yeemin 1986, Chornesky 1991, Shenk 1991). Skeletal fusion alone (without tissue fusion) has also been reported (filling: Potts 1976; cementation: Bak and Criens 1982; interdigitating skeletons: Chornesky 1991). This skeletal fusion offers some ecological benefits to interacting colonies, by contributing to the mechanical rigidity of fused partners as compared to separate fragments. Skeletal fusion also results in increased survivorship of cemented partners as compared to isolated ones (Bak and Criens 1982, Chornesky 1991). On the other hand, skeletal-fused colonies are not physiologically united like tissue-fused ones: In *Stylophora pistillata*, a microscopic gap of <10 µm demarcates both allogeneic tissues in a cemented pair of colonies (Rinkevich and Loya 1983a), and in *Hydractinia echinata*, an electron-dense fibrous barrier is formed between the tissues (Buss et al. 1985, Shenk 1991). Previous studies also revealed that the decalcification of skeletons in calcareous taxa always resulted in the separation of skeletal-fused allogeneic pairs (Bak and Criens 1982, Rinkevich and Loya 1983a, 1985). This is not the case where tissue fusion occurs. Tissue fusion in corals is always associated with skeletal fusion, which is mechanically more stable than in cementation of histoincompatible colonies (Bak and Criens 1982, Rinkevich and Loya 1983a, b, present study). Furthermore, such skeleton cementation is not only restricted to allogeneic or xenogeneic encounters, but also occurs between *Millepora dichtoma* and biologically inert objects (e.g. the clips which held the branches: Fig. 1d).

The lower mortality rates and the apparently better condition of compatible pairs, as compared with incompatible ones (Table 1), suggest again that the antagonistic responses between incompatible branches (probably not all expressed on the morphological level) may have significant costs (Ivker 1972, Rinkevich and Loya 1983b, 1985, Rinkevich and Weissman 1987). At present, we do not yet know whether two compatible allogeneic *Millepora dichtoma* fragments continue to coexist after fusion as a sectorial chimera, where cells of neither of the partners intermix, or whether cells migrate between genotypes. Intergenotypic migration of interstitial cells may lead to somatic cell parasitism of one genotype over the other by replacing the other partner's soma, or to germ cell parasitism by introducing foreign germ cells into the developing gonads (Buss 1982, Rinkevich and Weissman 1987). Furthermore, information on a possible correlation between genetic relatedness and allocompatibility is not available.

Bak and Criens (1982) interpreted tissue fusion between spatially-distinct *Acropora palmata* and *A. cervicornis* conspecifics to indicate clonemates, which were previously separated during colony fragmentation. Here, on the other hand, we have twice demonstrated that *Millepora dichtoma* colonies may fuse with other *specific allogeneic* entities. In the first case colony C fused with both colonies A and G. If indeed these three colonies (A, C and G) had been subclones belonging to only one genotype, colonies A and G should have fused as well. Instead, colony A repeatedly overgrew G in all eight studied replicates (Fig. 2 and an addition set of assays). A, C and G are therefore distinct genotypes. In the second example, the compatible colonies H and J (which fused in all eight assays) belong to two distinct genotypes, too, based on their extremely different positions in the network of colony hierarchies (Fig. 2). Colony J, the most superior colony, overgrew seven colonies, whereas H, the most inferior one, was overgrown by seven colonies. These results further support the conclusion that tissue compatibility assays are not an appropriate tool for assessing clonal identity in cnidarians (cf. Heyward and Stoddart 1985, Resing and Ayre 1985). In addition, these assays may provide some insight into the way in which *Millepora dichtoma* colonies distinguish self from nonself. As in *Hydractinia echinata* (Hauenschild 1954, 1956) and in botryllid ascidians (Oka and Watanabe 1960, Sabbadin 1962, Scofield et al. 1982), partial matching of allotypic determinants is enough for the recognition of self and the consequence fusion of allogeneic colonies.

In the field, tissue compatibility among nonclonemates may result in the formation of chimeric colonies. In *Hydractinia echinata* allogeneic partners in chimeras separted shortly after fusion (Shenk and Buss 1991). In *Millepora dichtoma*, in contrast, we did not record any evidence for separation in any of our 24 allogeneic chimeras following an observational period of 8 mo after fusion. It is therefore also possible that the non-reproducible directionality of primary overgrowth, recorded among replicates of seven allogeneic combinations (AE, AF, BC, FD, FG, IH, IJ; Fig. 2) resulted from sampling different regions of chimeric colonies.

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References

Bak, R. P. M., Criens, S. R. (1982). Experimental fusion in Atlantic *Acropora* (Scleractinia). Mar. Biol. Lett. 3: 67–72

Bak, R. P. M., Termaat, R. M., Dekker, R. (1982). Complexity of coral interaction: influence of time, location of interaction and epifauna. Mar. Biol. 69: 215–222

Beale, G. (1990). Self and nonself recognition in the ciliate protozoan *Euplotes*. TIG 6 (5): 137–139

Bosch, T. C. G., David, C. N. (1986). Immunocompetence in *Hydra*: epithelial cells recognize self-nonself and react against it. J. exp. Zool. 238: 225–234

Boschma, M. (1948). The species problem in *Millepora*. Zool. Verh., Leiden 1: 1–115

Boschma, M. (1949). Notes on the specimens of the genus *Millepora* in the collection of the British Museum. Proc. zool. Soc. Lond. 119 (3): 661–672

Buss, L. W. (1982). Somatic cell parasitism and the evolution of somatic tissue compatibility. Proc. natn. Acad. Sci. U.S.A. 79: 5337–5341

Buss, L. W., McFadden, C. S., Keene, D. R. (1984). Biology of Hydractiniid hydroids. 2. Histocompatibility effector system/competitive mechanism mediated by nematocyst discharge. Biol. Bull. mar. biol. Lab., Woods Hole 167: 139–158

Buss, L. W., Moore, J. L., Green, D. R. (1985). Autoreactivity and self-tolerance in an invertebrate. Nature, Lond. 313: 400–402

Chadwick-Furman, N. E., Rinkevich, B. (1994). A complex allorecognition in a reef building coral: delayed responses, reversals and nontransitive hierarchies. Coral Reefs (in press)

Chornesky, E. A. (1989). Repeated reversals during spatial competition between corals. Ecology 70: 843–855

Chornesky, E. A. (1991). The ties that bind: inter-clonal cooperation may help a fragile coral dominate shallow high-energy reefs. Mar. Biol. 109: 41–51

Cooper, E. L., Rinkevich, B., Uhlenbruck, G., Valembois, P. (1992). Invertebrate immunity: another viewpoint. Scand. J. Immun. 35: 247–266

Fishelson, L. (1973). Ecological and biological phenomena influencing coral-species composition on the reef tables at Eilat (Gulf of Aqaba, Red Sea). Mar. Biol. 19: 183–196

Hauenschild, C. (1954). Genetische und entwicklungsphysiologische Untersuchungen über Intersexualität und Gewebeverträglichkeit bei *Hydractinia echinata* Flem. Wilhelm Roux Arch. Entw Mech. Org. 147: 1–141

Hauenschild, C. (1956). Über die Vererbung einer Gewebeverträglichkeit-Eigenschaft bei dem Hydropolypen *Hydractinia echinata*. Z. Naturf. 11 b: 132–138

Heyward, A. J., Stoddart, J. A. (1985). Genetic structure of two species of *Montipora* on a patch reef: conflicting results from electrophoresis and histocompatibility. Mar. Biol. 85: 117–121

Hidaka, M. (1985). Tissue compatibility between colonies and newly settled larvae of *Pocillopora damicornis*. Coral Reefs 4: 111–116

Hildemann, W. H., Raison, R. L., Cheung, G., Hull, C. J., Akada, L., Okamoto, J. (1977). Immunological specificity and memory in a scleractinian coral. Nature, Lond. 270: 219–223

Ivker, F. B. (1972). A hierarchy of histo-incompatibility in *Hydractinia echinata*. Biol. Bull. mar. biol. Lab., Woods Hole 143: 162–174

Lange, R., Plickert, G., Müller, W. A. (1989). Histoincompatibility in a low invertebrate, *Hydractinia echinata*: analysis of the mechanism of rejection J. exp. Zool. 249: 284–292

Lasker, H. R., Coffroth, M. A. (1985). Vegetative reproduction, clonal spread, and histocompatibility in a Caribbean gorgonian. Proc. 5th. int. Coral Reef Congr. 4: 331–336 [Gabrié, C. et al. (eds) Antenne Museum-EPH, Moorea, French Polynesia]

Leddy, S. V., Green, D. R. (1991). Historecognition in the Cnidaria. In: Warr, G. W., Cohen, N. (eds.). Phylogenesis of immune functions. CRC Press, Boca Rato, Florida, p. 103–117

Lewis, J. B. (1989). The ecology of *Millepora*. Coral Reefs 8: 99–107

Logan, A. (1984). Interspecific aggression in hermatypic corals from Bermuda. Coral Reefs 3: 131–138

Loya, Y. (1972). Community structure and species diversity of hermatypic corals at Eilat, Red Sea. Mar. Biol. 13: 100–123

Müller, W. E. G., Maidhof, A., Zahn, R. K., Müller, I. (1983). Histocompatibility reactions in the hydrocoral *Millepora dichotoma*. Coral Reefs 1: 237–241

Oka, H., Watanabe, H. (1960). Problems of colony specificity in compound ascidians. Bull. mar. biol. Stn. Asamushi 10: 153–155

Potts, D. C. (1976). Growth interactions among morphological variants of the coral *Acropora palifera*. In: Mackie, G. O. (ed.) Coelenterate ecology and behavior. Plenum Press, New York, p. 79–88

Resing, J. M., Ayre, D. J. (1985). The usefulness of the tissue grafting bioassay as an indicator of clonal identity in scleractinian corals (Great Barrier Reef-Australia). Proc. 5th int. Coral Reef Congr. 6: 75–81 [Gabrié, C. et al. (eds.) Antenne Museum-EPHE, Moorea, French Polynesia]

Rinkevich, B., Frank, U., Bak, R. P. M., Müller, W. E. G. (1994). Alloimmune responses between *Acropora hemprichi* conspecifics: nontransitive patterns of overgrowth and delayed cytotoxicity. Mar. Biol. 118: 731–737

Rinkevich, B., Loya, Y. (1983 a). Intraspecific competitive networks in the Red Sea coral *Stylophora pistillata*. Corals Reefs 1: 161–172

Rinkevich, B., Loya, Y. (1983 b). Oriented translocation of energy in grafted reef corals. Coral Reefs 1: 243–247

Rinkevich, B., Loya, Y. (1985). Intraspecific competition in a reef coral: effects on growth and reproduction. Oecologia 66: 100–105

Rinkevich, B., Shashar, N., Liberman, T. (1993). Nontransitive xenogeneic interactions between four common Red Sea sessile invertebrates. Proc. 7th. int. Coral Reef Congr. Univ. Guam, Guam, p. 843–849

Rinkevich, B., Weissman, I. L. (1987). Chimeras in colonial invertebrates: a synergetic symbiosis or somatic- and germ-cell parasitism? Symbiosis 4: 117–134

Sabbadin, A. (1962). Le basi genetische della capacitá die fusione fra colonie in *Botryllus schlosseri* (Ascidiacea). Rend. Accad. Naz. Lincei. 32: 1031–1035

Salter-Cid, L., Bigger, C. H. (1991). Alloimmunity in the gorgonian coral *Swiftia excerta*. Biol. Bull. mar. biol. Lab., Woods Hole 181: 127–134

Scofield, V. L., Schlumpberger, J. M., West, L. A., Weissman, I. L. (1982). Protochordate allorecognition is controlled by a MHC like gene system. Nature, Lond. 295: 499–502

Shenk, M. A. (1991). Allorecognition in the colonial marine hydroid *Hydractinia* (Cnidaria/Hydrozoa). Am. Zool. 31: 549–557

Shenk, M. A., Buss, L. W. (1991). Ontogenetic changes in fusibility in the colonial hydroid *Hydractinia symbiolongicarpus*. J. exp. Zool. 257: 80–86

Shimizu, H., Sawada, Y. (1987) Transplantation phenomena in *Hydra*: cooperation of position-depended and structure-depended factors determining the transplantation results. Dev Biol. 122: 113–119

Toth, S. E. (1967). Tissue compatibility in regenerating explants from the colonial marine hydroid *Hydractinia echinata* (Flem.) J. Cell. Physiol. 69: 125–132

Vago, R. (1989). Mutual relationships in the biological association between the hydrocoral *Millepora dichotoma* and the *Savignun milleporum* barnacle (Cirripedia). MSc thesis, Bar Illan University, Ramat Gan (in Hebrew with English summary)

Wahle, C. M. (1980). Detection, pursuit, and overgrowth of tropical gorgonians by milleporid hydrocorals: Perseus and Medusa revisited. Science; N.Y. 299: 689–691

Weerdt, W. H. de (1981). Transplantation experiments with Caribbean *Millepora* species (Hydrozoa, Coelenterata), including some ecological observations on growth forms. Bijdr. Dierk. 51 (1): 1–19

Willis, B. L., Ayre, D. J. (1985). Asexual reproduction and genetic determination of growth form in the coral *Pavona cactus:* biochemical, genetic and immunogenic evidence. Oecologia 65: 516–525

Yamazato, K., Yeemin, T. (1986). Preliminary study on the inter- and intra-specific interactions among corals of Khang Khao islang, the Sichang islands, Gulf of Thailand. Galaxea 5: 163–174

Znidaric, D. (1981). Regeneration of autografts, isografts and xenografts of *Hydra*. Period. Biol. 83 (3): 295–299

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