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REPORT

Allorecognition maturation in the broadcast-spawning coral *Acropora millepora*

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Abstract Many sessile marine invertebrates discriminate self from non-self with great precision, but maturation of allorecognition generally takes months to develop in juveniles. Here, we compare the development of allorecognition in full-sibling, half-sibling and non-sibling contact reactions between newly settled juveniles of the broadcast-spawning coral *Acropora millepora* on the Great Barrier Reef (Australia). Absence of a rejection response showed that *A. millepora* lacks a mature allorecognition system in the first 2 months post-settlement. From thereon, incompatibilities were observed between juveniles, their level of relatedness (i.e. full-, half- and non-sibling status) governing the rate of allorecognition maturation. All contact reactions between non-siblings resulted in rejections by 3 months post-settlement, whereas the expression of allorecognition took at least 5 months between half-siblings and longer than 13 months for some full-siblings. Approximately 74 % of fused full-siblings ($n = 19$) persisted as chimeras at 11 months, thus maturation of allorecognition in this spawning coral appeared to be slower (>13 months) than in brooding corals (~4 months). We hypothesize that late maturation of allorecognition may contribute to flexibility in *Symbiodinium* uptake in corals with horizontal transmission, and could allow fusions and chimera formation in early ontogeny, which potentially enable rapid size increase through fusion.

Keywords Allorecognition · Immunity · Self–non-self-recognition · *Acropora millepora* · Corals

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Introduction

The ability to differentiate between self and non-self is a key feature of all living organisms. Precision in non-self-recognition and allorecognition mechanisms enables an organism to discriminate foreign from compatible genetic material, thereby providing the first line of defence against invading pathogens in both plants and animals (Nürnberger et al. 2004). Allorecognition comprises a series of events triggered by the contact between genetically different tissues, culminating in a rejection reaction in order to maintain the integrity of self (Grosberg 1988). However, sessile marine invertebrates like corals and ascidians are able to form entities consisting of tissues or cells of two or more genetically distinct individuals, that is, chimeras (Rinkevich and Weissman 1987; Puill-Stephan et al. 2009), indicating either a lack of precision in the self-recognition response or a delayed onset of precision early in ontogeny. Chimerism challenges many aspects of the purportedly accurate discrimination between self and non-self required

for immunocompetence, and also challenges the notion of genetic uniqueness within individuals and colonial organisms (Santelices 1999).

As adults, many sessile, modular marine invertebrates, such as sponges, cnidarians, bryozoans and ascidians, are able to discriminate self from non-self with great precision (Grosberg 1988). Because these marine invertebrates typically include asexual reproduction in their life histories, colonies originating from fragmentation or other asexual processes may come into contact with clone mates as they grow in size. Thus, allorecognition and non-self-recognition systems are essential for identifying colonies that are isogeneic (same species, same genotype), allogeneic (same species, different genotype) or xenogeneic (different species) and represent the first step leading to fusion or rejection reactions following the contact. Contacts between xenogeneic individuals invariably result in a rejection (or non-fusion) reaction, but contact between allogeneic or isogeneic individuals can lead to fusion, with allogeneic fusions resulting in the establishment of two or more genotypes within the same colony (Hart and Grosberg 1999). Because the allorecognition systems of adult colonial marine invertebrates generally discriminate between clone mates and non-clone mates effectively (Grosberg 1988), fusion between genetically different entities is commonly thought to be rare (Jackson 1986) and low numbers of chimeras typically occur in natural populations (Puill-Stephan et al. 2009). However, fusion of genetically distinct corals has been observed on multiple occasions (Heyward and Stoddart 1985; Resing and Ayre 1985; Willis and Ayre 1985). Furthermore, the occurrence of chimeras in natural populations of various colonial marine invertebrates (Sommerfeldt and Bishop 1999; Ben-Shlomo et al. 2001, 2008; Sommerfeldt et al. 2003; Rinkevich 2005; Puill-Stephan et al. 2009; Nozawa and Hirose 2011) and under experimental conditions (Amar et al. 2008; Puill-Stephan et al. 2012) indicates that their allorecognition systems at least occasionally allow the fusion of genetically non-identical entities.

The formation of chimeric entities in colonial marine invertebrates is believed to be more common during early ontogeny (Rinkevich 2004), as maturation of the allorecognition system may require a few months (or days depending on the organism). For example, a study using juveniles of the brooding corals *Seriatopora caliendrum* and *Seriatopora hystrix* revealed that fusions between grafted allogeneic colonies only occurred during the first 4 months post-settlement, suggesting that complete maturation of the allorecognition system requires about 4 months in these corals (Nozawa and Loya 2005). Lack of an allorecognition system in the early stages of post-larval settlement was also documented in four species of soft corals: *Nephthea sp.*, *Heteroxenia fuscescens*, *Parerythropodium fulvum* and *Clavularia hamra* (Barki et al. 2002). In these species, co-settlement of planula larvae resulted in high frequencies of allogeneic fusions, but these chimeras did not remain stable over long periods of time (up to 450 days) and many detrimental effects of fusion were noticed (such as the death of one or more partners, morphological resorption, slower growth). For the brooding coral *Stylophora pistillata,* three distinct stages in the development of the allorecognition system were defined, culminating in tissue separation or death of a partner when allorecognition matured by 4 months following settlement (Frank et al. 1997). Similarly, for the brooding coral *Pocillopora damicornis*, fusions were observed when juveniles originating either from the same colony or from different colonies were brought into contact from 7 days to 3 months after planulation (Hidaka et al. 1997). Contact reactions between juveniles originating from the same source colony (potentially full- or half-siblings) remained as chimeras for up to 7 months, whereas juveniles derived from different colonies fused in only a few cases and contact reactions subsequently resulted in non-fusion or incompatible fusion (Hidaka et al. 1997). Overall, fusions or rejections in these brooding corals appear to be linked to the timing of contact, that is, whether the contact happens before or after allorecognition systems are mature, but the outcomes of contacts were also strongly influenced by the relatedness (siblings vs. non-siblings) of different entities in the contact interaction.

The maturation of allorecognition in broadcast-spawning corals, the spatially dominant and numerically most abundant group of reef corals, is still poorly investigated. Broadcast-spawning corals typically acquire their algal endosymbiont, *Symbiodinium,* through uptake from the environment (horizontal uptake, Harrison and Wallace 1990), in contrast to brooding corals that generally acquire symbionts maternally (vertical transmission). This represents a major difference in life history strategies that could influence the allo- and non-self-recognition systems of corals. Furthermore, allorecognition maturation with respect to fine-scale genetic relatedness, that is, the capacity to distinguish between half-siblings and full- or non-siblings, has not been investigated for any coral species. Here, we assess the maturation of allorecognition within *Acropora millepora,* a widespread and abundant broadcast-spawning coral species on the Great Barrier Reef. Specifically, we investigate whether the outcomes of contact reactions between juveniles of this coral vary with different levels of relatedness, time and age. Research into the maturation of allorecognition may also enhance the understanding of the immune system of corals, providing insights into factors contributing to their vulnerability in relation to environmental disturbances and disease.

Materials and methods

Coral species and study sites

This project investigated maturation of allorecognition in *Acropora millepora*, a broadcast-spawning coral that is both abundant and ubiquitous on the Great Barrier Reef, Australia. This species is currently the best characterized coral at the molecular level, and its husbandry is relatively well developed. Thus, *Acropora millepora* represents a good study species for allorecognition experiments involving early ontological stages.

Mature colonies of *A. millepora* were collected from two field sites (Nelly Bay, Magnetic Island, and South West Pelorus Island), both located in the central Great Barrier Reef in Australia, prior to the predicted spawning events of October 2007 at Magnetic Island and November 2007 at Pelorus Island (Willis et al. 1985; Babcock et al. 1986). Colonies from Magnetic Island were transferred to the Australian Institute of Marine Science and those from Pelorus Island to the Orpheus Island Research Station for spawning and gamete collection. Colonies were maintained in 1,000-L tanks supplied with running 1 µm filtered sea water (FSW) at 28.5 °C. The genotype of each coral colony collected was determined prior to spawning based on analyses of 3 microsatellite loci (van Oppen et al. 2007) to ensure that colonies were genetically distinct and to avoid crosses between clone mates.

Rearing larvae

On the day of spawning, colonies were isolated in individual 70-L aquaria filled with 1 µm filtered sea water (FSW) and kept isolated until they had finished spawning. Gametes from six colonies were collected and mixed in separate 70-L aquaria to produce four crosses (A, B, C and D in Fig. 1). These crosses were replicated at both sites. Juveniles from these four crosses were used to create contact reactions between corals that differed in kinship level, that is, full-sibling, half-sibling and non-sibling contact reactions (Fig. 1).

Gametes were allowed to fertilize for at least 1.5–2 h, after which a small subset of eggs was sampled for microscopic confirmation of fertilization and initiation of embryogenesis. Embryos were cleaned by performing three consecutive water changes, which involved draining ~90 % of the water from the bottom and slowly filling from the top. Embryos from each cross were then transferred into separate 500-L tanks supplied with running FSW at 28.5 °C in a temperature-controlled room and kept at a density of approximately one larva per mL. Embryos were checked microscopically in order to assess their development until ~48 h after fertilization, when the fully ciliated planula larva stage was reached. Four days after spawning, when swimming larvae had become elongated and had started to search the substratum for suitable settlement sites, the bottom of each tank was covered with underwater paper (previously rinsed and soaked in FSW for 24 h) as settlement surfaces.

Establishing contact reactions

Ten days after spawning, contact reactions (Fig. 1, Table 1) were set up between coral juveniles settled on underwater writing paper by cutting out settled juveniles and pasting them next to each other on plastic tiles using MrSticky's® underwater glue. Contacts were established so that juveniles were either just touching (immediate contact), 2 mm away from each other or 5 mm away from each other. Juvenile pairings established with increasing distances between coral recruits were designed to create contacts at a series of time points as the juveniles grew, to test whether contact reactions (fusion or rejection) changed with the age of juveniles. Contacts between full-, half- and

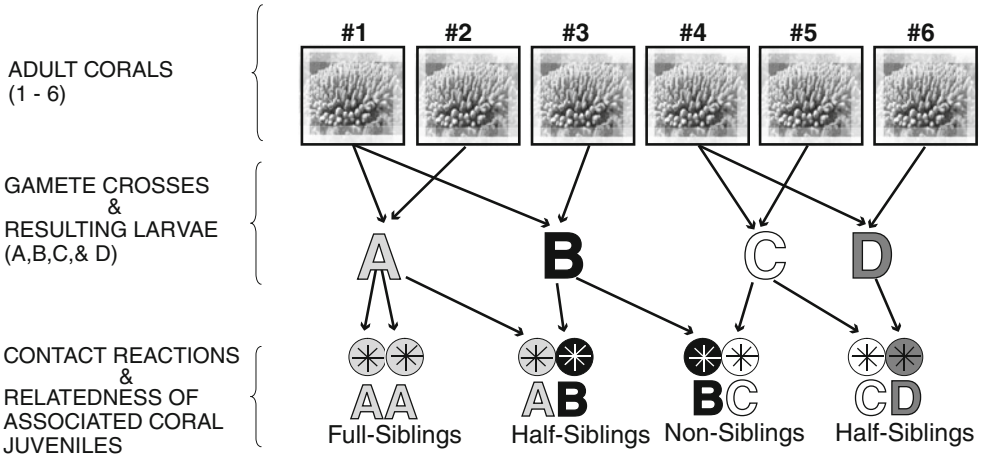

Fig. 1 Schematic diagram showing how gametes from different colonies (identified by numbers 1–6) were crossed to produce larval groups (*A*, *B*, *C* and *D*) and how contact reactions were set up between juveniles that differed in their kinship. Note that contact reactions between non-siblings *AC*, *AD*, *BD* and full-siblings *BB*, *CC* and *DD* are not illustrated here

Table 1 Number of surviving pairs, 1 month after setting up contact reactions between juveniles of the broadcast-spawning coral *Acropora millepora*

Larvae	A			B			C			D		
	Immediate contact	2 mm	5 mm	Immediate Contact	2 mm	5 mm	Immediate contact	2 mm	5 mm	Immediate contact	2 mm	5 mm
A	5	1*		3*			1		1	4	1*	
	1*											
B				8	1		1	1*	1*	3	1	1
C							9			1		3
D										8		
										1*		

Distances between juveniles at the beginning of the study were 0 mm (immediate contact), 2 mm and 5 mm. Numbers with an asterisk (*) represent contacts between juveniles originating from Pelorus Island

non-siblings were established between juveniles that had settled solitarily. Juveniles that had settled in aggregations (two or more juveniles settled adjacently) in the settling tanks were designated as immediate contact reactions between full-sibling juveniles. Because larvae from different crosses were maintained and settled separately, immediate fusion at settlement occurred only between full-siblings reared in the same tank.

Contact reactions between juveniles were named according to the relatedness of the paired juveniles; thus AA, BB, CC and DD represented pairings of juveniles that were full-siblings, that is, each pair comprised juveniles originating from the same two parent colonies (see Fig. 1 for relatedness between juveniles). Contact reactions named AB and CD represented pairings between half-siblings, that is, the two juveniles in contact pairings shared one parent. Contact reactions named AC, BC, AD and BD represented pairings between non-siblings, that is, the two juveniles in each contact pairing had different parents. Ten replicate contact reactions were established for each type of sibling pairing at each of the three distances (immediate, 2 and 5 mm).

Eleven days after spawning, the laboratory-reared juvenile corals in contact reaction pairings were placed in the field at 5 m depth in Nelly Bay (Magnetic Island), an inshore reef (Babcock and Mundy 1996; Anthony et al. 2004) with a gentle slope down to approximately 10 m. The plastic tiles were skewered on rods through a hole in the centre of the tiles, with spacers (2–3 cm long) between each tile. The rods were suspended between two star pickets, which had been driven into dead substratum on the reef, so that tiles were maintained in a vertical orientation to minimize the accumulation of sediment on tile surfaces. Tiles were labelled, tagged and photographed prior to the deployment on the reef.

Assessing contact reactions

In order to investigate the fate of contact reactions between coral juveniles, tiles were monitored and photographed 10 days after spawning, every month up to May 2008 (i.e. for 6 and 5 months post-settlement for corals from Magnetic Island and Pelorus Island, respectively) and then on the 30th October 2008 (i.e. almost 12 and 11 months post-settlement for corals from Magnetic Island and Pelorus Island, respectively). The experiment was terminated 13 months post-settlement. Tiles were kept in the field, at 5 m depth, in Nelly Bay (Magnetic Island).

The outcomes of contact reactions were scored microscopically as fusions (F), identified when tissues appeared to be continuous across the contact area and new polyps appeared along the contact margin (Fig. 2a, e), or rejections (R), which were characterized by discontinuity of tissues along the line of contact (i.e. no tissue fusion and/or no addition of new polyps, Fig. 2c) and a white line (sometimes very thin, Fig. 2b) along the contact zone. The rejection category was equivalent to the non-fusion categories of Hidaka et al. (1997), and Nozawa and Loya (2005). A third type of contact reaction, incompatible fusion, has been described by Hidaka et al. (1997) and Nozawa and Loya (2005) and characterized as apparent fusion of tissues of paired corals; however, the presence of a distinct white border zone along the contact area clearly separates the two juveniles. For simplicity and because tissues were clearly incompatible with a distinct white border zone along the contact area during our observations, we included all non-fusions in the rejection category. In order to maximize the number of replicates surviving through to the end of the study, we did not sample any juveniles for genetic analysis or histological confirmation of fusions.

Analysis

Statistical tests were performed using the software JMP 9. A non-parametric Kaplan–Meier survival test was performed for comparisons of mortality among the three different sibling groups in contact throughout the study period.

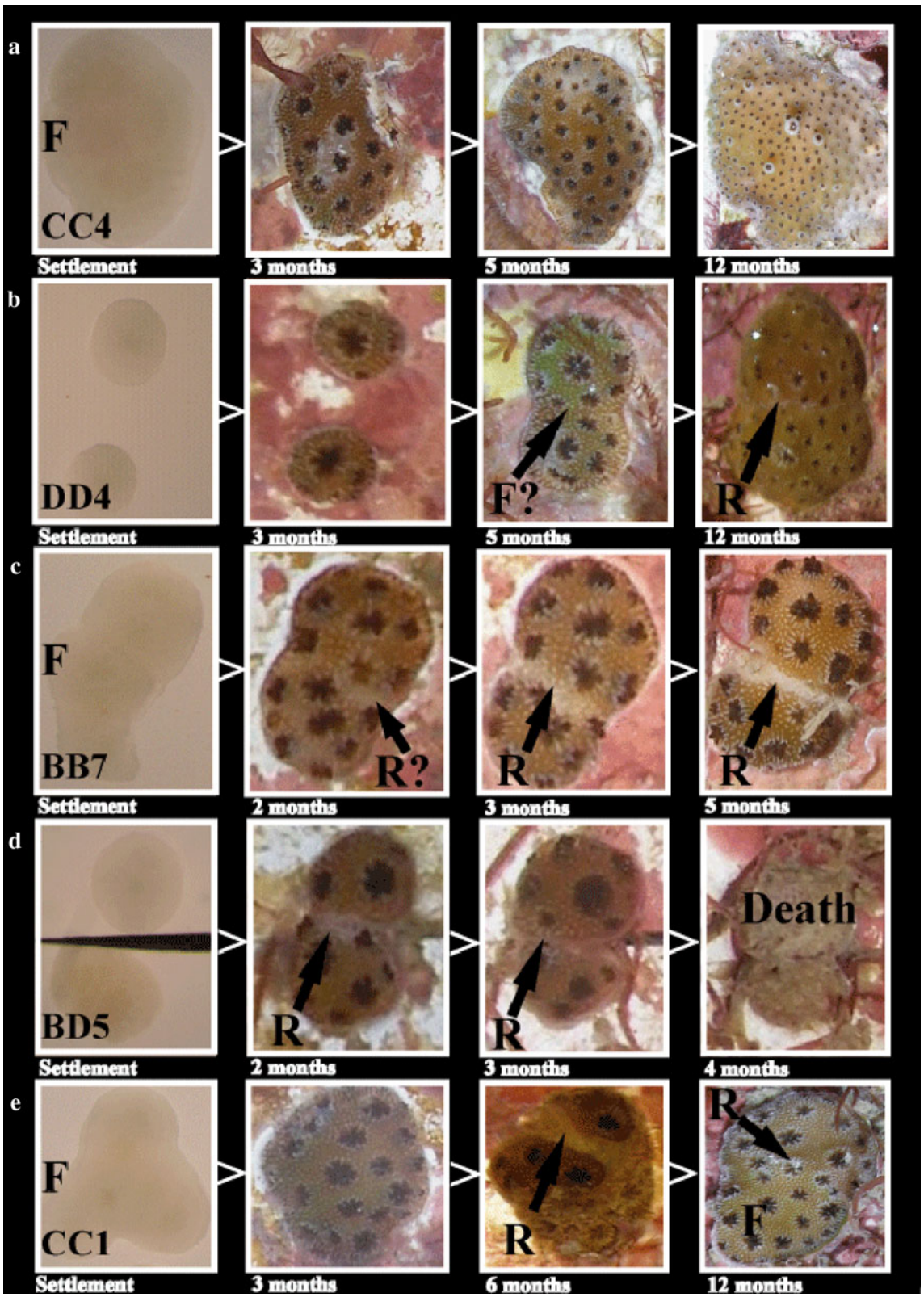

Fig. 2 Examples of temporal patterns in the outcomes of contact reactions between juveniles of *Acropora millepora* for full-sibling (**a**, **b**, **c**, **e**) and non-sibling (**d**) contact reactions. (F: fusion, R: rejection, and Death)

Results

Contact reactions between *A. millepora* juveniles

From the 300 contact reactions originally set up for each juvenile group (Magnetic and Pelorus), only 48 Magnetic Island and 9 Pelorus Island juveniles survived after 1 month and could therefore be monitored from then on (Table 1).

Every contact reaction monitored between non-siblings resulted in a rejection at some point, with 14 out of 15 of non-sibling interactions (i.e. >93 %) displaying rejection reactions shortly after initial contact (Fig. 3). AD4 was the only non-sibling pairing that initially resulted in a fusion

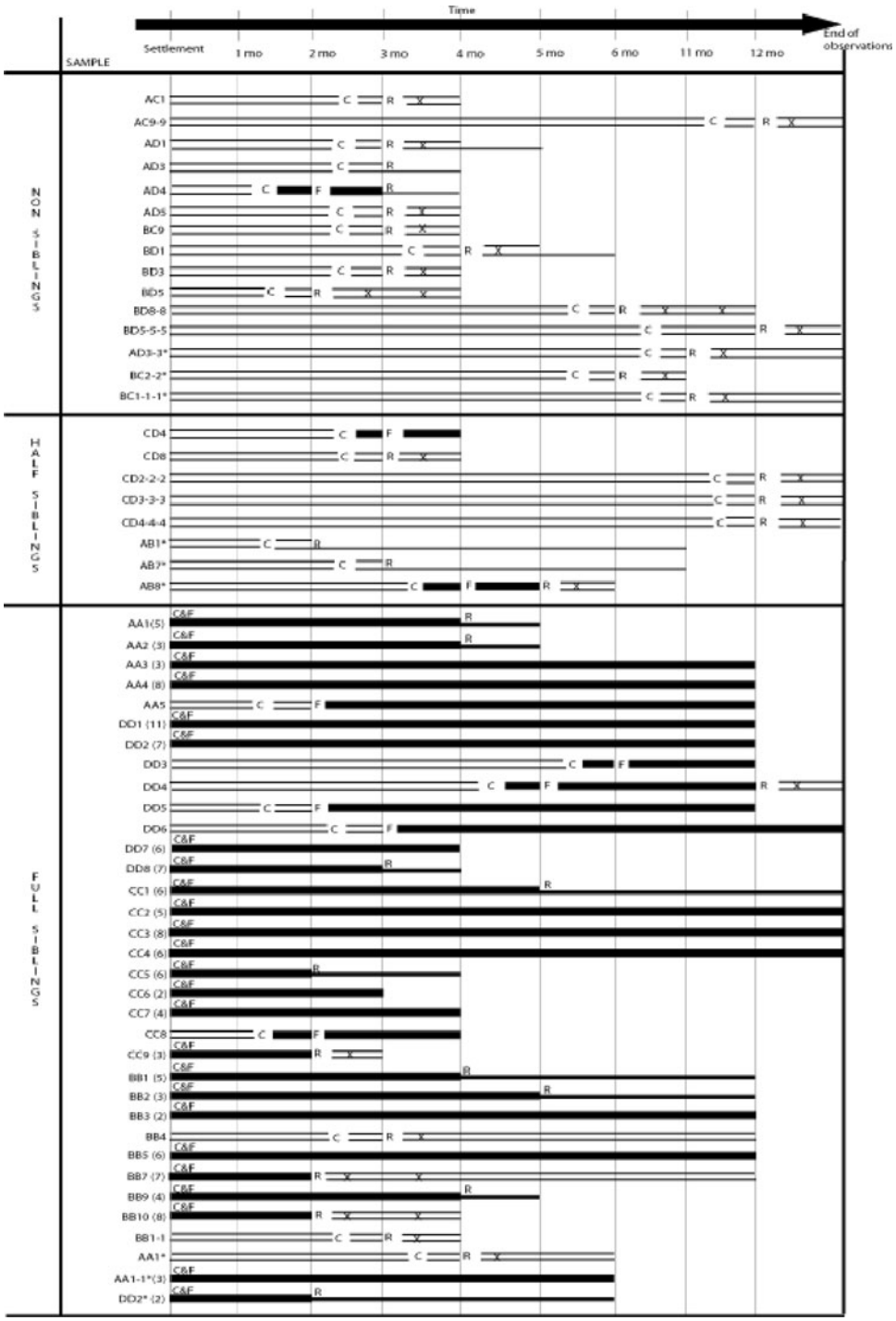

Fig. 3 Observation of contact reactions between *A. millepora* juveniles from settlement until 13 months post-settlement. *Bold lines* represent fused juveniles. *Medium bold lines* represent fused juveniles within a colony that has displayed signs of rejection. *Thin lines* represent non-fused juveniles. *Thin lines* separated by a *cross* represent non-fused juveniles following rejection. *End of lines* represent either the death of the corals or the end of the observation period. C: timing of first contact between different juveniles, F: timing of observed fusion, R: timing of observed rejection. When juveniles were fused at settlement, numbers of fused recruits are indicated in *brackets*. For example, the contact reaction between full-sibling s *AA1*, has 5 recruits in contact, hence *AA1(5)*. Juveniles marked with an asterisk (*) originated from Pelorus Island

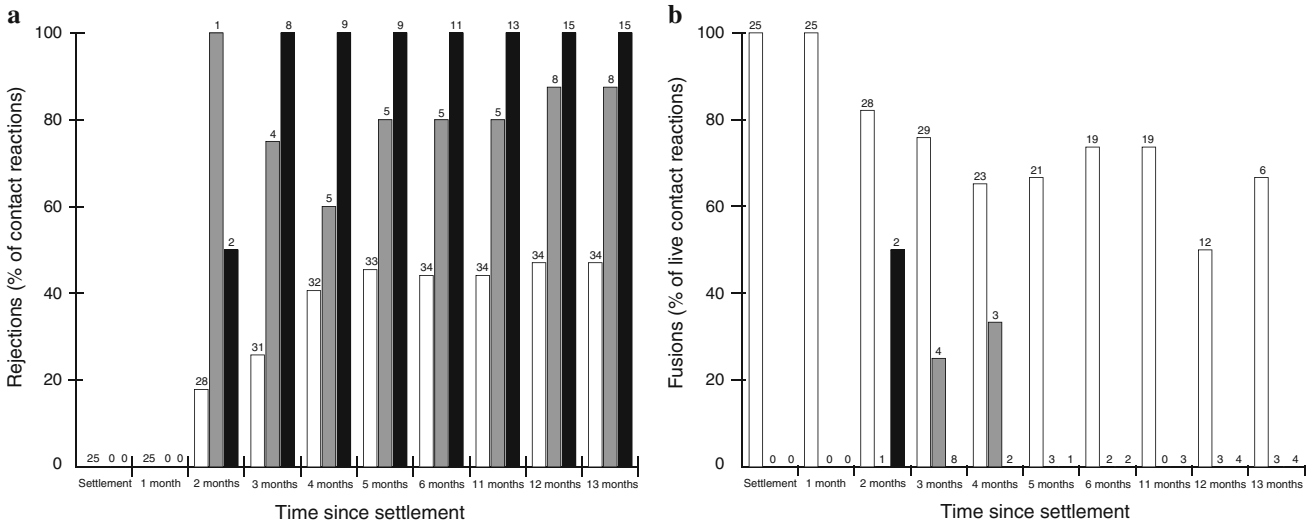

Fig. 4 Total per cent of rejections (**a** in % of live and dead corals in contact) or fusions (**b** in % of corals alive in contact) for contacts between full-sibling (*white*), half-sibling (*grey*) and non-sibling (*black*) *A. millepora* juveniles, from settlement up to 13 months. *Numbers* of contacts are indicated above each histogram

reaction (1 fusion after initial contact out of 15 contact reactions; i.e. 7 %), which was visible at approximately 2 months post-settlement (Fig. 3). However, this initial fusion was reversed 1 month later (3 months after settlement), with signs of rejection observed 3 months post-settlement (AD4 on Fig. 3).

At approximately 1.5 months post-settlement, B grew into contact with D (replicate 5: BD5) and A grew into contact with D (replicate 4: AD4). The first signs of rejection were observed 2 months post-settlement in pair BD5 (Figs. 2d, 3). Then, from 3 months post-settlement until the end of the experiment, 100 % of non-sibling juveniles in contact displayed signs of rejection (Fig. 4a).

Six out of 8 contacts monitored between half-siblings (i.e. 75 %) resulted in rejection reactions after initial contact (Fig. 3). Only two out of the eight (i.e. 25 %) half-sibling pairings resulted in fusion, one at 3 months and one at 4 months post-settlement. However, in one case (CD4), the fused colony died within 1 month, potentially before a rejection reaction was visible, and in the other case (AB8*, juveniles marked with an asterisk * originated from Pelorus Island), signs of rejection were visible within a month of observing the initial fusion reaction (Fig. 3). Overall, seven out of 8 contacts between half-sibling juveniles (i.e. 87.5 %) ultimately displayed signs of rejection during the 13 month monitoring period (Fig. 4a).

Fusion was far more prevalent between full-siblings than between half-sibling or non-sibling pairings. Most of the fusions (25 of 31 fusions) occurred at settlement, when two or more juveniles settled in aggregations. In addition, 6 out of 9 contacts happening after the first month of the monitoring period (i.e. ~67 %) resulted in fusion following the initial contact (Fig. 3). Only 3 out of 9 full-sibling contact interactions (i.e. 33 %) did not result in fusion after the initial contact (AA1*, BB4, and BB1-1, Fig. 3). However, 13 out of 31 fused colonies (i.e. ~40 %) eventually showed signs of rejection. The first signs of rejection within fused aggregations (i.e. fused at settlement) were observed at 2 months post-settlement (for pairs BB7, BB10, CC5, CC9 and DD2*; Figs. 2c, 3). Nevertheless, at 11 months post-settlement, 14 out of 19 full-siblings in contact and alive (i.e. ~73 %) were still fused and did not show signs of rejection (Fig. 4b), and 4 out of 6 colonies alive were still fused at 13 months post-settlement (i.e. ~66 %).

Overall (Magnetic and Pelorus Island juveniles combined), mortality rates within the first month post-settlement reached 94 % ($n = 240$), 93 % ($n = 120$) and 86 % ($n = 240$) for non-, half- and full-siblings, respectively. Mortality rates from 1 month post-settlement onwards until the end of the study reached 73 % for non-siblings (11 deaths out of 15 contacts), 62 % for half-siblings (5 deaths out of 8 contacts) and 65 % for full-sibling pairings (22 deaths out of 34 contacts). Although non-siblings displayed the highest mortality rates, no significant differences were found among the three different sibling groups in contact throughout the study period (Kaplan–Meier survival test: log rank $X^2 = 0.455$, DF = 2; Wilcoxon $X^2 = 0.892$, DF = 2).

Discussion

This study indicates that juveniles of the broadcast-spawning coral *Acropora millepora* lack precision in allorecognition early in ontogeny, with maturation of the

allorecognition system beginning at approximately 2 months post-settlement. Increasing numbers of rejections in full-sibling contact reactions, from 18 % at 2 months (5 out of 28 contact reactions) to approximately 47 % at 12 months (16 out of 34 contact reactions), suggests that development of the allorecognition system is gradual in this coral species (Fig. 4a). In combination with findings of stepwise maturation of allorecognition systems by 3–4 months in juveniles of the brooding corals *Stylophora pistillata* (Frank et al. 1997), *Seriatopora hystrix and Seriatopora caliendrum* (Nozawa and Loya 2005), our results highlight an emerging pattern of delayed maturation of allorecognition systems in juvenile corals, with much longer delays potentially occurring in broadcast-spawning corals. As also highlighted by Hidaka et al. (1997), the level of genetic relatedness between juveniles strongly influences the outcome of contact reactions in the first few months after settlement, with fusion generally occurring when juveniles are full-siblings, whereas rejection generally occurs between unrelated juveniles.

Our study investigates allorecognition maturation at a fine scale of genetic relatedness, that is, at the level of distinguishing between half-siblings and full- or non-siblings, by comparing the outcomes of contact reactions at the three kinship levels. Six out of nine full-sibling pairings (i.e. 67 %) of *A. millepora* resulted in fusion when juveniles first came into contact, including contact reactions that were initiated between 5 and 6 months post-settlement. In contrast, only two out of eight half-sibling pairings (i.e. 25 %) and one out of fifteen non-sibling pairings (i.e. 7 %) resulted in fusion at first contact. Two of these fused half- and non-sibling colonies survived for more than 2 months following fusion but in both cases, initially fused colonies showed the signs of rejection within 1 month of fusion. In contrast, fusions persisted in half of the full-sibling pairings that were still alive at 12 months ($n = 6$ juveniles out of 12 alive, or 17 % of all full-sibling pairings), indicating that once fused, there may be selective advantages in maintaining chimeric colonies when partners are full-siblings. The comparatively low levels of fusion in half- and non-sibling contact reactions indicate that relatedness strongly influences the outcome of the contact.

An obvious advantage of fusion is the ensuing rapid increase in juvenile size, as supported by evidence that early fusions lead to more rapid increases in size than growth rate alone can provide for solitary juveniles (Raymundo and Maypa 2004; Puill-Stephan et al. 2012). Although rapid size increase is an obvious consequence of fusion, no significant differences in survival and no robust advantage could be attributed to fusion and chimerism (Puill-Stephan et al. 2012).

Although not significant, the highest mortality rates in our study were observed in non-sibling contact reactions, compared to half- or full-siblings. These differences in the levels of mortality among relatedness groups might reflect lower levels of intergenotype conflict in contacts involving closely related siblings. Such findings could provide support for an eventual selective advantage in fusions between closely related siblings and may also underlie the persistence of chimeras in adult populations of this species (Puill-Stephan et al. 2009). However, our results should be carefully interpreted due to the low replication in the number of contact reactions and high mortality rates of juveniles, which reached over 90 % during the first month of the experiment across all sibling groups. Further research would be required to elucidate the benefits or disadvantages of fusion and chimera formation.

This study supports recent evidence of a slower maturation of allorecognition in spawning corals (Nozawa and Hirose 2011) in contrast with the allorecognition system maturation at 4 months in brooding corals, as documented by Frank et al. (1997), and Nozawa and Loya (2005).

However, a recent study on the brooding coral *Stylophora pistillata* recorded fusions after 1 year (Amar and Rinkevich 2010), but juveniles in this study may have been genetically identical given that some pocilloporids are known to have the ability to produce asexual larvae. Additionally, authors concluded that coral juveniles' genomes may be largely shared, because the genetic diversity of these populations may have been reduced as a consequence of chronic anthropogenic impacts (Amar and Rinkevich 2010).

Nozawa and Loya (2005) suggested that complete maturation of the allorecognition system required at least 4 months in *Seriatopora caliendrum* and *S. hystrix*, because fusions between grafted allogeneic colonies were only observed during the first four months post-settlement. Nevertheless, the self–non-self-recognition system of *Seriatopora* functions to some extent during the first four months post-settlement, given that juveniles rejected genetically distant tissues more consistently than closely related tissues (Nozawa and Loya 2005). A study of contact reactions between allogeneic juveniles revealed stepwise progression in the maturation of the juvenile allorecognition system in the brooding coral *Stylophora pistillata* and identified three distinctive stages in the maturation process (Frank et al. 1997). When juveniles were younger than 2 months, almost all allogeneic colonies fused to form morphologically stable chimeras. Then, for contacts that occurred between 2 and 4 months post-settlement, fusion was transitory and ended by tissue separation or death of a partner at the age of 4 months. After 4 months, no fusions between allogeneic tissues were recorded, indicating the maturation of the allorecognition system.

On the other hand, maturation of allorecognition appears to be slower in spawning corals. Indeed, in our study on the broadcast-spawning species *A. millepora,* indiscriminate

fusion of juveniles occurred in the first month post-settlement, and although some signs of rejection were observed as early as 2 months in contact reactions involving non-, half- or full-siblings, fusions were still observed 6 months post-settlement. Moreover, many full-sibling fusions showed no signs of rejection even at 13 months, which is when the study ended. Similarly, allogeneic fusions were observed in natural conditions, 2–3 years post-settlement in the coral *Echinophyllia aspera* (Nozawa and Hirose 2011). Consequently, our results indicate that the development of allorecognition in at least one spawning coral species is slower than rates typically reported for brooding corals. Cases of sibling juveniles remaining fused after 13 months highlight the possibility that allorecognition systems in broadcast-spawning corals enable closely related genotypes to form stable chimeras.

Differences in the onset of allorecognition between spawning and brooding corals may be related to divergent symbiont acquisition strategies. One of the differences between most broadcast-spawning and brooding corals is the acquisition of algal symbionts from the environment by larvae or recent recruits of spawning corals, while brooded larvae acquire *Symbiodinium* through vertical transmission from their mother. The uptake of *Symbiodinium* by coral larvae and juveniles during early ontogeny is relatively non-selective. During the first few months post-settlement, juveniles of *Acropora tenuis* and *A. millepora* are able to take up various *Symbiodinium* types, regardless of the type present in parental colonies (Abrego et al. 2009). Although there is no proof that recognition of allogeneic coral tissues and xenogeneic *Symbiodinium* cells involves the same immune pathways, the non-selectivity of *Symbiodinium* uptake in the first few months post-settlement provides further support that acroporid corals lack a mature non-self-recognition system during this time. After an initial flexible uptake (Little et al. 2004), corals become dominated by one symbiont type (Abrego et al. 2009), reflecting the possible maturation of non-self-recognition. Therefore, the lack of an efficient non-self-recognition system in the first few months may be a factor contributing to initial flexibility in symbiont uptake.

While it is clear that adult corals need efficient allo- or non-self-recognition systems to respond to various external assaults, such as diseases, bacteria and competition with other animals or plants, the reasons for the apparent lack of efficient allorecognition early in ontogeny are less obvious.

The formation and persistence of entities involving associations of genetically different juveniles (i.e. chimeras) could represent either a case of allorecognition "failure" or an acceptance and co-habitation (i.e. tolerance) of closely related genotypes. Hence, there may have been selective pressure for the mature alloimmune system of corals to tolerate the persistence of fusions between compatible or closely related genotypes within stable chimeras, as a consequence of benefits associated with rapid size increase through fusion (Raymundo and Maypa 2004) and possibly with chimeric vigour. Evidence that chimeras occur and persist in wild populations of *A. millepora* on the Great Barrier Reef, Australia (Puill-Stephan et al. 2009), and in the coral *Echinophyllia aspera* in Kochi, Japan (Nozawa and Hirose 2011), provide further support that stable chimeras could offer selective advantages and might explain fusion events observed between different adult corals of the same species in self-recognition bioassays in earlier studies (Willis and Ayre 1985).

In summary, we observed that juveniles of *A. millepora* lack an efficient allorecognition system in early ontogeny. Signs of rejection between conspecific juveniles of the same age were only observed after 2 months post-settlement, even if juveniles had been in contact for over 1 month (such as full-sibling aggregations fused since settlement and then showing first rejection signs only at 2 months). The level of genetic relatedness strongly influenced the outcome of contact reactions between juvenile corals, as all non-siblings rejected each other from 3 months post-settlement onwards, whereas full-siblings could still fuse 6 months post-settlement and remained fused after 1 year. The initial absence and slow maturation of allorecognition and possibly of self-recognition may be beneficial for spawning corals, as it enables conspecifics to settle together, fuse and form chimeras in order to increase in size more rapidly than through growth alone, and it may also facilitate flexibility in symbiont uptake. However, potential advantages associated with rapid growth and flexibility in symbiont acquisition may increase the vulnerability of juveniles to external stressors, such as pathogens. Hence, further research is required to elucidate how the early immune and self-recognition systems of corals respond to these challenges.

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