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NOTE

Spawning and fertility of F_1 hybrids of the coral genus *Acropora* in the Indo-Pacific

Naoko Isomura[1] · Kenji Iwao[2] · Masaya Morita[3] · Hironobu Fukami[4]

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Abstract The role of hybridization through multi-specific synchronous spawning in the evolution of reef-building corals has been discussed since the 1990s, particularly for the genus *Acropora*. However, F_1 hybrids have been reported as common in only one case in the Caribbean, with no evidence of mechanisms that would allow continuous reproduction of the hybrids. In this study, we report for the first time the fecundity of two F_1 hybrid colonies produced experimentally from two Indo-Pacific species, *A. intermedia* and *A. florida*. These F_1 hybrids spawned at the same time as the parental corals. Backcrossing and F_1 hybrid crossing were successful in both directions. Furthermore, more than 90% self-fertilization was achieved in an F_1 hybrid, although it was negligible in the parental corals. While it is possible that the F_1 hybrid was a chimera, these results suggest that some products of interspecific hybridization may persist as the offspring of self-fertilizing F_1 hybrids.

Keywords Hybrid · Self-fertilization · Fecundity · Scleractinia

Introduction

Corals in the genus *Acropora*, which contains the greatest number of species (ca. 150) and greatest morphological diversity among scleractinian corals (Wallace 1999), reproduce once a year during multi-specific synchronous spawning events (Harrison 2011). Reproductive isolation among many species is maintained during these spawning events, despite the fact that unfertilized eggs and sperm from many different species occur concurrently at the surface (Willis et al. 2006). However, in vitro experiments have shown that some species can cross-fertilize (Hatta et al. 1999; Willis et al. 2006), and interspecific hybridization has therefore been considered to occur occasionally in the field.

High potential for natural hybridization within *Acropora* has in fact been shown by several molecular studies and crossing experiments in vitro (Willis et al. 2006; Isomura et al. 2013). In the Indo-Pacific, Willis et al. (2006) reared F_1 hybrids between *A. millepora* and *A. pulchra* that were characterized by a morphology intermediate to their parents, although they died before sexual maturation. Coral colonies with morphologies intermediate of other species have also occasionally been observed in the field (Isomura et al. 2013), but there is no direct evidence that they are true natural F_1 hybrids. Conversely, in the Caribbean, molecular analysis confirmed that one of the three *Acropora* species, *A. prolifera*, is an F_1 hybrid between the two

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✉ Hironobu Fukami
hirofukami@cc.miyazaki-u.ac.jp

[1] Department of Bioresources Engineering, National Institute of Technology, Okinawa College, 905 Henoko, Nago-City, Okinawa 905-2192, Japan

[2] Akajima Marine Science Laboratory, Zamami, Okinawa 901-3311, Japan

[3] Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Sesoko, Motobu, Okinawa 905-0227, Japan

[4] Department of Marine Biology and Environmental Science, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

others (*A. cervicornis* and *A. palmata*) and exhibits a colony morphology intermediate to those of its parental species (Vollmer and Palumbi 2002).

F_1 hybrids are expected to play an important role in the evolution of corals. They are potentially fitter than their parental species in certain environmental settings, which would promote range expansion and adaptation to new environments (Willis et al. 2006; Fogarty 2012). In order to predict the ecological fitness and persistence of F_1 hybrids in the field, it is, however, necessary to determine whether they are capable of reproducing sexually either by crossing with other hybrids, or by backcrossing with their parental species.

Among Indo-Pacific *Acropora* species, *A. intermedia* and *A. florida* are an ideal species pair to investigate hybridization processes in corals because they exhibit clear differences in colony morphology, with the first species displaying an arborescent form and the second a thick "bottle-brush" branching form. These two species are also known to successfully hybridize during in vitro trials (fertilization rate of 2.7–98.7%; Isomura et al. 2013).

In this study, we aimed at clarifying whether F_1 hybrids of *A. intermedia* and *A. florida* could potentially persist in the wild through sexual reproduction. To do so, we documented the timing of spawning and assessed their fertility using in vitro crossing experiments. The experiments consisted of intraspecific crosses, backcrossing with parental species, crosses between hybrids, and self-fertilization trials.

Materials and methods

Growth form of hybrids and purebreds

F_1 hybrid embryos between *A. intermedia* and *A. florida* were produced by in vitro crossing experiments in Akajima, Okinawa, Japan (26°12′N, 127°17′E), in 2007 (Isomura et al. 2013). We performed two crosses, INTflo and FLOint, to compare their growth form and to produce a large number of F_1 hybrid embryos. The first cross (INTflo) consisted of ~5000–10,000 eggs from one *A. intermedia* colony and sperm from three *A. florida* colonies, and the second cross (FLOint) consisted of ~35,000 eggs from one *A. florida* colony and sperm from three *A. intermedia* colonies. The coral rearing process from embryos to juveniles was performed as described by Omori and Iwao (2014). F_1 hybrid larvae from each of the two hybrid types (1100 larvae of INTflo and 12,400 larvae from FLOint) as well as purebred larvae (5000 larvae of each parental species) were kept in individual 100-L tanks with eight 10 × 10 cm ceramic plates that were previously conditioned in the sea for 1 month. Settlement rates after 1 week were 56.2, 7.4, 18.1, and 5.1% for INTflo, FLOint, *A. florida*, and *A. intermedia*, respectively. All ceramic plates with recently settled corals (INTflo, FLOint, and purebreds for each parental species) were placed, together with juveniles of the top shell snail *Trochus niloticus* (a grazer of macroalgae) (Omori 2005), inside an 8-mm mesh cage (30 cm height × 55 cm width × 80 cm length) suspended at a depth of 3.4 m (2 m above the seafloor) in the bay of Akajima; the corals have been maintained within the cage since 2007 to prevent the dispersion of F_1 hybrids into the field. By June 2014, only two mature F_1 hybrid colonies of similar sizes (INTflo and FLOint) remained in the cage, because most of the hybrids and purebreds were removed in 2011 due to limited space. Only a few purebreds were tentatively transplanted to the field in Akajima at that time.

Spawning observations and crossing experiments

Because *Acropora* colonies are known to be mature at 4 yr old in Akajima (Iwao et al. 2010), we assessed gamete maturity annually in the two remaining F_1 hybrids starting in 2011. Spawning observations and crossing experiments (intraspecific crossing of the parents, backcrossing, crosses between F_1 hybrids, and selfing) were performed in Akajima on 16 June 2014. Five days before the predicted spawning date, these two F_1 hybrid colonies were removed from the cages and placed in a flow-through water tank in the laboratory. Similarly, fragments (~20 cm long) of *A. intermedia* and *A. florida* colonies (five colonies each) were collected in the field in Akajima 3–5 d prior to predicted spawning date and placed in the same flow-through aquarium system. All of these wild colonies in the field were tagged with plastic plates to identify individuals for the following year. Spawning observations and crossing experiments were performed following Isomura et al. (2013). The final sperm concentration used in the crossing experiments was adjusted to approximately 10^6 sperm mL^{-1}. For each cross, 100–300 eggs were used. Fertilization rates were calculated by counting the number of fertilized and unfertilized eggs from a total of 100 eggs collected randomly from each cross 3 h after mixing of the sperm and eggs. Fertilized embryos from each cross were placed in a polypropylene cup filled with 50 mL of filtered seawater (10 µm pore size) until they developed into planula larvae.

Additional crosses similar to those described above were performed on 1 June 2015, when INTflo gametes were backcrossed with three colonies of each parental species (same colonies as those sampled in 2014). Lastly, self-compatibility in FLOint was assessed on 5 July 2015, by performing self-fertilization experiments with various sperm dilutions (10^4–10^7 sperm mL^{-1}).

Results and discussion

The morphology of each single remaining mature colony of the F_1 hybrids (FLOint and INTflo) (Fig. 1e, f) was closer to that of their maternal species than the paternal species (Fig. 1a, b), whereas purebreds showed colony forms similar to those of their parents (Fig. 1c, d). The INTflo colony exhibited relatively few short branches and a nearly arborescent form, whereas the FLOint colony exhibited multiple short branching, similar to bottle-brush branching. Vollmer and Palumbi (2002) discussed maternally derived morphology in hybrids of *Acropora* species and suggested a cytoplasmic influence on this trait. Although the morphology of these hybrids might have been affected to some extent by the fact that they were grown inside cages, it is apparent that they exhibit a growth form intermediate to that of their parental species. Furthermore, the gross morphology of the FLOint colony seemed to be very similar to that of the suspected hybrid between *A. intermedia* and *A. florida*, namely the only colony of its kind that was found in the field to date (Isomura et al. 2013). This suggests that natural hybrids between *A. intermedia* and *A. florida* are rarely produced in the field.

The two F_1 hybrids spawned for the first time on 16 June 2014 (Fig. 1f). Notably, FLOint started spawning in conjunction with its maternal species *A. florida* at 3 h after sunset, and then 15 min later, INTflo started spawning in conjunction with its maternal species *A. intermedia*. While FLOint released more than 500 bundles, INTflo released approximately 30 bundles. Fecundity of the two hybrids

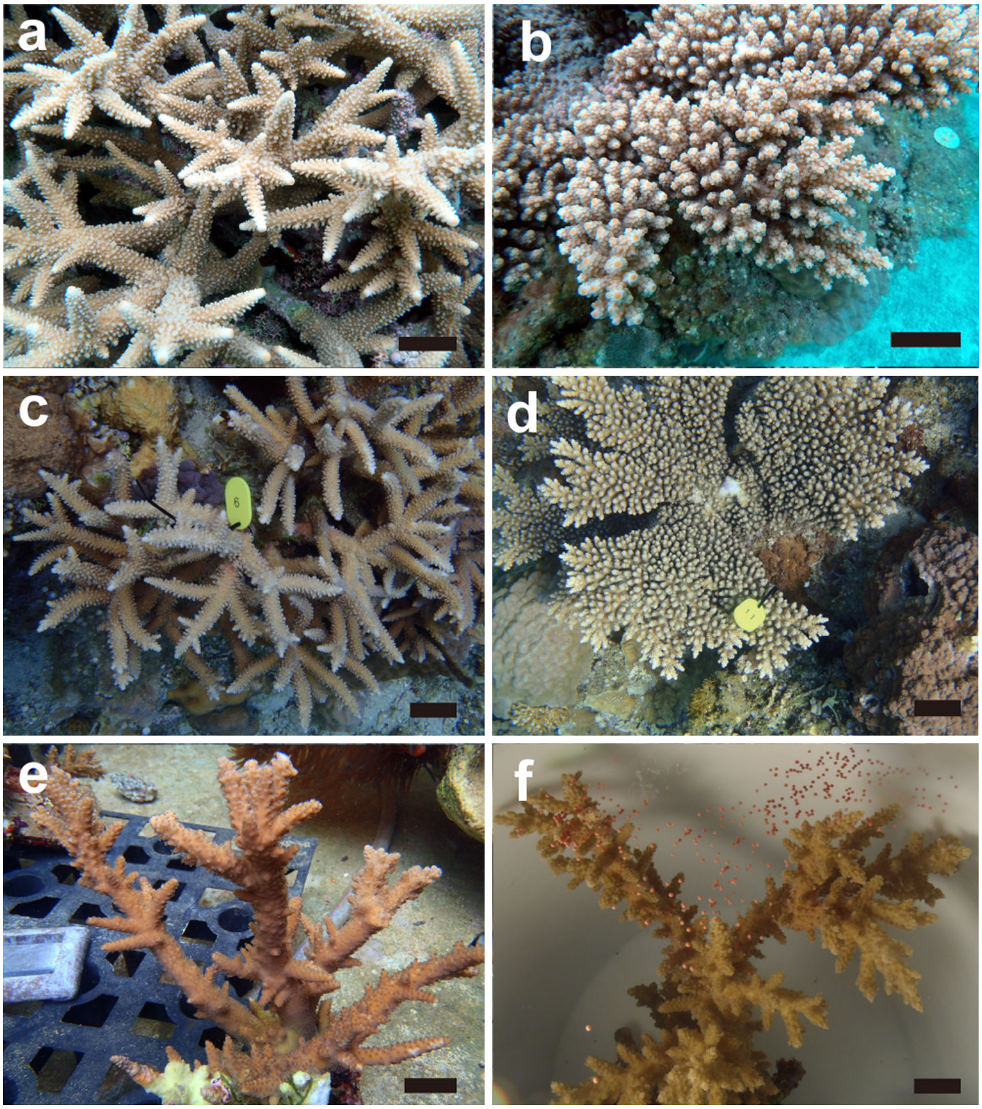

Fig. 1 F_1 hybrids and parental species of *Acropora* in Akajima, Okinawa, Japan: **a** *A. intermedia*, **b** *A. florida*, **c** a 7-yr-old purebred of *A. intermedia*, **d** a 7-yr-old purebred of *A. florida*, **e** F_1 hybrid INTflo, **f** spawning F_1 hybrid FLOint. *Bar* = 5 cm

was similar in 2015 with INTflo again releasing approximately 30 bundles on the same date as maternal species, and with FLOint releasing approximately 500 sperm–egg bundles. However, spawning of FLOint was delayed by 1 month (5 July 2015) compared to all other colonies we observed in this study. This is the first observation of spawning in F_1 hybrids. However, these results are derived from a single colony of each F_1 hybrid type; further experiments with larger numbers of F_1 hybrid colonies are needed to confirm their reproductive characteristics.

Data from all crossing experiments performed in 2014 and 2015 are available in Electronic Supplementary Material (ESM) Fig. S1, which shows that fertilization rates between pairs were consistent between years. Figure 2 shows a summary of the results of all crossing experiments conducted in 2014 and the crossings of INTflo in 2015. Two new findings were evident from these experiments: first, backcrosses of both F_1 hybrids with their parental species were successful along with crosses between the two F_1 hybrids; and second, high self-fertilization rates were found in FLOint but not in INTflo and the two parental species.

FLOint gametes successfully backcrossed with each parental species regardless of whether egg or sperm of FLOint were used, whereas INTflo gametes did not backcross in one cross (INTflo sperm × *A. florida* eggs), although we could not test for other crosses because of the small number of bundles. Furthermore, F_1 hybrid crossing (INTflo × FLOint) was successful with high fertilization rates in both directions of eggs and sperm (Fig. 2).

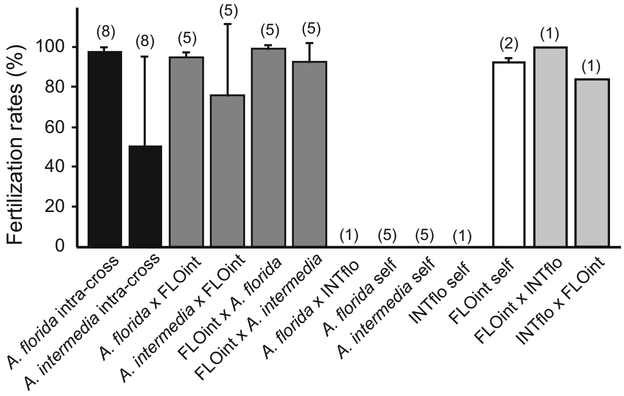

Fig. 2 Average fertilization rates in crossing experiments conducted in 2014 using the F_1 hybrids and parental species of *Acropora* and in 2015 using INTflo. Intraspecific crosses of the parental species are shown in *black*. Backcrosses are shown in *dark gray*. Self-fertilization is shown in *white*. The crossing between F_1 hybrids is shown in *gray*. Cross combinations indicate species of eggs first, followed by species of sperm (i.e., eggs × sperm). *Error bars* represent standard deviations. The total number of crosses is shown in *parentheses* above the *bar*

Selfing with extremely high fertilization rates (>90%) was observed in FLOint and was confirmed by independent experiments conducted over 2 yr (Fig. 2; Table 1). This is the first substantial record of selfing in the genus *Acropora*. Notably, selfing with high fertilization rates occurred under a low sperm density (10^4 sperm mL^{-1}), even though the optimal sperm concentration is approximately 10^{5-6} sperm mL^{-1} in *Acropora* (Nozawa et al. 2015). In contrast, INTflo did not exhibit selfing (0%) under a low sperm density (10^4 sperm mL^{-1}) (Fig. 2), although we could not test this at higher sperm densities because of the small number of available bundles. Selfing may occur when the self-incompatibility (SI) system of gametes is disrupted. In ascidians, self-recognizing proteins named “themis” are located on the sperm (S-themis A and B) or vitelline envelope (V-themis A and B) (Sawada et al. 2014). The *S-themis* and *V-themis* genes are genetically closely linked, reside at the same loci in the chromosome, and contribute to rejection of self-sperm (Sawada et al. 2014). The presence of themis-like proteins in coral gametes has not yet been reported, but the high rates of selfing observed in FLOint imply that the SI system of this hybrid was somehow disrupted. On the other hand, successful self-fertilization in FLOint may result from chimerism. Puill-Stephan et al. (2009) found that 2–5% of *Acropora* colonies in the field are chimeras, and this proportion may be even higher in artificially bred colonies due to high density of larval settlement on artificial substrates compared to the field. Further genetic analyses are required to confirm the mechanism by which this FLOint hybrid successfully self-fertilizes, whether it is through disruption of the SI system or via chimerism.

The majority of intraspecific, backcrossed, and selfed embryos and F_2 hybrid embryos developed normally into planula larvae. There were no differences in development time and no significant differences in planula survivorship among crossing pairs (ESM Fig. S2), suggesting that no post-zygotic isolating barrier exists at the planula stage. Together, these findings illustrate that F_1 hybrids of Indo-Pacific *Acropora* species such as FLOint could survive and persist as populations or species via backcrossing and selfing, although populations may be small considering that no direct evidence of their existence in the field has been found thus far. Some rare Indo-Pacific *Acropora* species that occupy atypical or non-parental niches have been suggested to be of hybrid origin based on genetic evidence (Richards et al. 2008). However, the reproductive mechanism by which their populations are maintained remains unknown. As reported for plants (Barrett 2003), self-fertilization may be a process by which such hybrid populations are maintained in the wild. Selfing is generally not favored in species that typically outcross because self-fertilized progeny often have lower fitness than their

Table 1 Self-fertilization rates with different sperm densities using a F_1 hybrid, FLOint, which was produced from eggs of *Acropora florida* and sperm of *A. intermedia*

Sperm density (sperm mL^{-1})	10^4	10^5	10^6	10^7
Number of crosses	1	3	3	1
Mean fertilization rate (%) ± SD	96.0	97.1 ± 1.3	95.9 ± 2.5	96.0

outcrossed conspecifics (Knowlton and Jackson 1993). The current study shows that the hybrids of two *Acropora* species are capable of selfing, and that the resulting embryos had equal early development times and larval survival rates to those of outcrossed embryos. Although it is necessary to know how selfing occurs in the presence of sperm from non-self mates, selfing may play an important role in maintaining the hybrid population and facilitating speciation in the species-rich genus *Acropora*.

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