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Reproductive Isolation among *Acropora* Species (Scleractinia: Acroporidae) in a Marginal Coral Assemblage

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Nuwei Vivian Wei, Hernyi Justin Hsieh, Chang-Feng Dai, Carden C. Wallace, Andrew H. Baird, and Chaolun Allen Chen (2012) Reproductive isolation among *Acropora* species (Scleractinia: Acroporidae) in a marginal coral assemblage. *Zoological Studies* **51**(1): 85-92. Hybridization was proposed as being an important source of evolutionary novelty in broadcast-spawning reef-building corals. In addition, hybridization was hypothesized to be more frequent at the periphery of species' ranges and in marginal habitats. We tested the potential for hybridization in 2 ways: observations of the time of spawning and non-choice interspecific fertilization experiments of 4 sympatric *Acropora* species in a non-reefal coral assemblage at Chinwan Inner Bay (CIB), Penghu Is., Taiwan. We found that colonies of more than 1 species rarely released gametes at the same time, thus limiting the opportunities for cross-fertilization in the wild. On the few occasions when different species released gametes in synchrony, interspecific fertilization in experimental crosses was uniformly low (the proportion of eggs fertilized ranged 0%-4.58% with a mode of 0%), and interspecific-crossed embryos ceased development and died within 12 h after initially being fertilized. Ecological and experimental analyses indicated that reproductive isolation exists in these 4 *Acropora* species even though they have the opportunities to spawn synchronously, suggesting that hybridization is not very frequent in this marginal coral habitat at CIB.
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Key words: *Acropora*, Hybridization, Synchronous spawning, Marginal coral community.

The Indo-Pacific scleractinian genus *Acropora* is one of the most comprehensively studied coral groups in terms of evaluation of hybridization as a mechanism contributing to species richness. *Acropora* is a diverse genus of more than 100 species, up to 76 of which can occur in sympatry (Wallace 1999). Furthermore, 35 sympatric *Acropora* species were reported to release gamete bundles within a 2-h period during multi-species spawning events on the Great Barrier Reef (GBR) (Willis et al. 1985, Babcock et al. 1986), and similar multi-species spawning events occur in most specious coral assemblages (Baird et al. 2009), thus providing an opportunity for interspecific breeding (Willis et al. 2006, Fukami et al. 2008). Willis et al. (2006) in a review of artificial cross-fertilizations of Indo-Pacific *Acropora*, estimated that 45% of the species examined could form hybrids, although in most crosses, only a few eggs were fertilized. They concluded that the capacity to hybridize is common in Indo-Pacific *Acropora* (Willis et al. 2006). Similarly, while there

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are only 3 endemic *Acropora* in the Caribbean, morphological and genetic data suggest that *A. prolifera* is a hybrid of *A. cervicornis* and *A. palmata* (van Oppen et al. 2000, Vollmer and Palumbi 2002), and all 3 species are believed to be capable of interbreeding (reviewed in Willis et al. 2006).

Although hybridization has received a great deal of attention as a potential mechanism for speciation in *Acropora*, there is evidence to suggest that hybridization in nature is less common than *in vitro*. For example, fine-scale temporal differences in gamete release and differences in the time taken for egg/sperm bundles to break up are sufficient to prevent cross-fertilization among species that spawn on the same night (Fukami et al. 2003, Wolstenholme 2004). Indeed, some species that spawn as little as 1.5 h apart are genetically distinct (van Oppen et al. 2002, Fukami et al. 2004). Furthermore, when eggs are offered a choice of both intra- and interspecific sperm, hybrid embryos are very rarely produced (Willis et al. 2006). Similarly, sperm display less activity in response to heterospecific eggs than to conspecific ones. These observations suggest that corals possess many prezygotic barriers that reduce the chance of hybridization in the field. In addition, population genetic analyses of *A. hyacinthus* and *A. cytherea* from allopatric populations indicated that these 2 species constitute distinct entities, despite producing a high proportion of hybrid embryos in artificial crosses and extensively sharing alleles according to phylogenetic analyses (Marquez et al. 2002a b). Such cases may represent incipient species (i.e., of very recent origin) in which reproductive barrier are incomplete. Alternatively, they may represent cases of incomplete lineage sorting in reproductively isolated species that still retain different degrees of ancestral polymorphism.

The extent of hybridization or genetic isolation may vary among different biogeographical regions and also as a function of the number of congeneric species participating in multi-species spawning events. Willis et al. (2006) speculated that when many species spawn simultaneously, there will be strong selection for efficient gamete recognition; whereas at sites where gametes of fewer species interact, selection may be less stringent. In other words, they predicted that rates of hybridization would be more frequent at the periphery of coral species' distribution ranges, where numbers of species are lower than on the GBR and Okinawa, where numbers of sympatric *Acropora* species are relatively high (Willis et al. 2006).

In the present study, the reproductive properties of 4 sympatric *Acropora*, *A. muricata*, *A. hyacinthus*, *A. humilis*, and *A. valida* were investigated in Chinwan Inner Bay (CIB), Penghu I., Taiwan. Penghu I. is surrounded by a subtropical non-reefal coral community (Chen 1999) with low coral diversity compared to tropical reefs (Hsieh 2008). CIB is a semi-enclosed bay where the coral assemblage at depths of 2-6 m is dominated by these 4 *Acropora* species (Hsieh 2008). Synchronous spawning of scleractinian corals occurs from late Apr. to early June, depending on the lunar phase (Chen unpubl. data). Using observations of spawning times and artificial crosses of these 4 species, we examined the hypothesis that hybridization is more frequent in a marginal habitat at the periphery of these species' distribution range.

MATERIALS AND METHODS

Study site

Field observations of coral spawning and cross-fertilization experiments were conducted between 2002 and 2005 at the Marine Biology Research Center (MBRC), a facility of the Taiwan Fisheries Research Institute, located adjacent to the coral assemblage in CIB, Penghu I., Taiwan (23°31'N, 119°33'E) in the Taiwan Strait. The coral assemblage in the CIB has developed atop volcanic rocks and is characterized by the dominance of Acroporiidae in shallow waters, including *A. muricata*, *A. hyacinthus*, *A. valida*, *A. humilis*, and *Montipora cactus* (Hsieh 2008). Spawning of these species has been monitored since 1999 by tagging colonies and field observations, revealing that most scleractinian corals in CIB release gamete bundles around the full moon in late Apr., early May, late May, or early June, depending on the lunar phase (Table 1, Chen et al. unpubl. data).

Tank observations of spawning and crossing experiments

Five days before the predicted date of a spawning event, 4-6 colonies of *A. muricata*, *A. hyacinthus*, *A. valida*, and *A. humilis* were collected, placed in individual buckets, and incubated in tanks with through-flow seawater. At the time of collection, separate colonies of each species sampled were tagged in the field.

When spawning in the tanks was observed, the tagged colonies were checked the following day to determine whether or not the corresponding field colonies had also released gametes. On the date of the predicted spawning night, the seawater was switched off to avoid contamination by sperm from the ocean. Time of sunset, colony setting, and bundle release were recorded.

Cross-fertilization experiments were conducted following Willis et al. (1997). Gamete bundles were collected immediately following spawning. Eggs and sperm were separated using plankton mesh sieves, and eggs were washed in at least 10 changes of sperm-free seawater. Sperm density was estimated, and suspensions of 10^6 sperm/ml were prepared. This concentration produced the best results in non-choice crossing experiments (Willis et al. 1997). Breeding trials were followed by the instruction matrix described in Willis et al. (1997). Intraspecific, interspecific, and self-fertilization trials consisted of approximately 100 eggs added for 2 h of spawning to the diluted sperm stock in 25-ml screw-cap glass vials. Controls were conducted using washed eggs incubated in sperm-free water in order to verify that the seawater used was sperm-free.

Vials containing gametes were placed in baskets with floats and attached to a jetty at the MBRC in order to mimic the conditions of natural wave agitation and ambient sea temperatures. Approximately 4 h after crossing, vials were retrieved, and the ratio of fertilization was determined. The percent fertilization was calculated from the number of embryos among the total number of propagules counted. Only embryos that had reached the 4- or 8-cell stage (Miller and Ball 2000) were scored as having been successfully fertilized. Additional counts were made at 8-12 h after the initial fertilization, to confirm continuous embryo development until at least the "prawn-chip" stage (Miller and Ball 2000). Observations were continued at 24, 48, 72, and 96 h to record the development of embryos into planula larvae.

RESULTS

Spawning day and time

The spawning day of *Acropora* spp. in CIB, Penghu Is., ranged from 7 d before to 9 d after the full moon in Apr. and May (Table 1). In 2002, 1 colony of *A. muricata* and 1 colony of *A. humilis* spawned in the tank 3 d before the full moon in Apr., followed by 1 colony of each of the 3 species

Table 1. Spawning day, relationship to the full moon (-, day before the full moon; +, day after the full moon), sunset times of spawning days, and beginning of spawning times of 4 sympatric *Acropora* spp. in Chinwan Inner Bay, Penghu, Taiwan in 2002-2005. Number of colonies observed in the laboratory are shown in parentheses. Blanks indicate that spawning was not observed in those species in the laboratory. Sunset times were retrieved from the database of the Central Weather Bureau, Taiwan

Species	2002						2003		
Date	24 Apr. (-3)	25 Apr. (-2)	27 Apr. (0)	28 Apr. (+1)	29 Apr. (+2)	May 2 (+5)	Apr. 20 (+4)	Apr. 23 (+7)	May 23 (+8)*
Sunset	18:23	18:23	18:23	18:26	18:26	18:26	18:21	18:21	18:35
A. muricata	20:00 (1)		20:15 (1)	20:00 (1)	20:05 (3)	20:30 (4)	20:30 (6)	20:45 (4)	20:30 (4)
A. humilis	20:15 (2)	20:00 (1)		20:30 (1)	20:30 (2)	21:00 (2)	20:40 (4)		20:45 (6)
A. valida		20:00 (1)	20:00 (1)	20:15 (1)		20:30 (2)	20:30 (2)	20:40 (3)	20:30 (2)
A. hyacinthus		20:00 (1)	21:00 (1)		20:10 (1)	20:30 (3)		20:50 (3)	20:50 (6)

Species	2004		2005						Bundle setting time	Interval after setting
Date	May 11 (+8)	May 13 (+10)	May 1 (+8)	May 2 (+9)	May 15 (-7)*	May 16 (-6)	May 19 (-3)	May 20 (-2)		
Sunset	18:31	18:33	18:26	18:26	18:33	18:33	18:35	18:35		
A. muricata	20:40 (3)	20:50 (2)	20:40 (1)	20:40 (3)	20:30 (3)		21:00 (3)	21:00 (2)	19:10-19:50	30-70 min
A. humilis		21:00 (2)					21:30 (1)	21:15 (2)	19:00-19:50	35-60 min
A. valida	20:40 (3)			20:50 (3)		20:40 (4)	21:30 (3)		19:00-20:00	35-80 min
A. hyacinthus	20:50 (3)	21:00 (1)						21:30 (3)	19:00-19:50	45-100 min

releasing gamete bundles 2 nights before the full moon, the night of full moon, and 1 night after the full moon. More than 1 colony of each species spawning synchronously was observed for 3 species on the 2nd night after the full moon. However, synchronous spawning of 4 species was only observed on the 5th night after the full moon. In 2003, 3 *Acropora* spp. were observed to release gamete bundles in the tanks on the 4th and 7th nights after the full moon. All 4 species spawned on the 8th night. In 2004, only 3 species spawned in the tanks on the 8th and 10th nights after the full moon in May. In 2005, spawning was even less synchronous with 1 and 2 species respectively spawning on the 8th and 9th nights after the full moon in Apr., and 3 species spawning 2 d before the full moon in May. No synchronous spawning was observed among any of the 4 species in 2005 (Table 1). All tagged colonies that released gamete bundles in the tank also released gamete bundles in the field.

With all 4 species, gamete bundles appeared in the polyp mouth between 19:10 and 20:00 in 2002-2005 (Table 1). Bundle release occurred between 20:00 and 21:30, i.e., 30 to 100 min after sunset. A 10-30-min time delay was observed between species that spawned on the same night. In most cases, *A. muricata* was the 1st species to spawn, and *A. humilis* was the last during nights of synchronous spawning.

Crossing experiments

The results of cross-fertilization experiments are summarized in table 2. Intraspecific crosses (excluding selfing) consistently produced high fertilization rates, ranging from 94.73% in *A. muricata* to 99.33% in *A. hyacinthus*. Selfing occurred at a very low rate (0.63%-0.89%) except with *A. humilis* (5.97%). Interspecific fertilization was consistently very low, ranging from 0% with *A. humilis* sperm × *A. hyacinthus* eggs to 4.58% in the cross of *A. valida* sperm × *A. hyacinthus* eggs. All interspecific fertilizations were associated with high standard errors, suggesting that successful fertilization rates were highly variable among the crosses, and the mode was 0%. While intraspecific-crossed embryos continued to develop into "prawn-chip" stage at 12 h after initial fertilization, all interspecific-crossed embryos had died by 2 d, and no further embryo development stage was observed in the vials. All intraspecific-crossed embryos had successfully developed to swimming planula larvae by 96 h.

DISCUSSION

In the present study, results of spawning dates, times, and interspecific crossing experiments indicated that reproductive isolation

Table 2. Fertilization trials within and between species of 4 sympatric *Acropora* spp. The mean fertilization percentages of all individual crosses conducted between 2002 and 2005 in Chinwan Inner Bay were averaged for each combination, and values are shown with the standard error. Values for the intraspecific crosses are the averages of both reciprocal combination of eggs and sperm. *n* is the number of colonies used in the trials

Egg	Sperm				Controls (no sperm)
	A. muricata	*A. humilis*	*A. valida*	*A. hyacinthus*	
A. muricata (*n* = 21)	94.73 ± 9.15 (112/112)	0.18 ± 1.04 (0/63)	0.13 ± 0.50 (0/105)	0.08 ± 0.38 (0/90)	0.00 ± 0.00 (0/63)
A. humilis (*n* = 9)	1.25 ± 2.97 (1/59)	97.28 ± 4.35 (24/24)	1.74 ± 4.11 (1/21)	1.88 ± 4.36 (4/48)	0.00 ± 0.00 (0/27)
A. valida (*n* = 15)	2.11 ± 7.03 (5/105)	0.00 ± 0.00 (0/21)	95.53 ± 5.98 (78/78)	1.37 ± 4.80 (2/46)	0.00 ± 0.00 (0/36)
A. hyacinthus (*n* = 11)	1.47 ± 3.33 (4/81)	1.26 ± 2.88 (2/48)	4.58 ± 10.57 (7/46)	99.33 ± 1.55 (54/54)	0.00 ± 0.00 (0/33)
Cross of the same colony	0.77 ± 2.65 (1/60)	5.97 ± 8.20 (6/27)	0.63 ± 1.28 (0/36)	0.89 ± 1.67 (0/33)	

exists among the 4 dominant *Acropora* species, and hybridization is likely to be very rare among *Acropora* in CIB, Penghu I., a non-reefal marginal coral community in Taiwan (Chen 1999, Hsieh 2008).

Prezygotic isolation: species diversity and spawning times

Acropora species diversity and spawning patterns in CIB greatly differ from those on the GBR, Australia and at Akajima I., Okinawa, Japan where species diversity is relatively high and many species spawn in synchrony (Willis et al. 1985, Babcock et al. 1986, Hayashibara et al. 1993). Up to 76 *Acropora* species occur in sympatry, and 35 of them have been recorded to release gamete bundles within 2 h of each other during spawning events on the GBR (reviewed in Willis et al. 2006). Similar patterns were observed at Akajima I., Okinawa, where 10-12 of 35 *Acropora* species were recorded to spawn on the same nights (Hayashibara et al. 1993, Hatta et al. 1999). In contrast, at CIB and other adjacent islands, *A. muricata*, *A. valida*, *A. humilis*, and *A. hyacinthus* are the dominant species in the coral assemblage (Hsieh 2008). Spawning of these 4 species was observed from the 7th night before to the 9th night after a full moon depending on the month and year (Table 1). In most cases, 2 or 3 species spawned 1.5-2 h after sunset, and synchronous spawning of all 4 species was only observed on 1 night each in 2002 and 2003, suggesting that *Acropora* spawning at CIB is less synchronous than at the GBR and Okinawa. Although the time of gamete bundle release was similar among species (0-30 min), successful interspecific fertilization rates remained low. In contrast to the hypothesis of Willis et al. (2006), reproductive barriers caused by gamete recognition appear to be strong even in regions such as Taiwan where there are few congeneric *Acropora* species and where gametes rarely interact because of year-to-year variations in lunar nights of spawning. A similar pattern of non-synchronous gamete release by sympatric *Acropora* species was also observed at a high-latitude coral community at Otuski, Kochi, Japan (Takemura et al. 2007). At a high-latitude coral community in Shirahama, Wakayama, Japan, spawning times of 2 *A. solitaryensis* ecomorphs differed by 13-18 min, but there was a significantly low in vitro fertilization rate between ecomorphs. In contrast, 1 ecomorph, the arborescent *A. solitaryensis*, can cross with *A. pruinosa* with a significantly high fertilization rate *in vitro* (Suzuki and Fukami 2011). However, *A. pruinosa* spawned approximately 1 h earlier than both ecomorphs of *A. solitaryensis in vivo* at Shirahama; thus, it is unlikely that these 2 high-latitude *Acropora* would hybridize in the field. Overall, when the ecological aspects of spawning are considered, such as fine-scale differences in the timing of gamete release and bundle breakup (Wolstenholme et al. 2003, Wolstenholme 2004), as well as species differences in sperm aging, dispersal, and dilution of gametes (Levitan et al. 2004), interspecific fertilization might occur very rarely in the field.

Postzygotic isolation: low interspecific crossing rates, zygotic morality, and hybrid inviability

Even though there is potential for *Acropora* species to spawn synchronously and interspecifically fertilize *in vitro*, postzygotic isolation mechanisms, such as gametic incompatibility, zygotic morality, hybrid inviability, hybrid sterility, and hybrid breakdown, still work against hybridization frequently occurring among species.

In the case of *Acropora* at CIB, gametic incompatibility, zygotic mortality, and hybrid inviability might prevent hybridization from occurring. First, interspecific fertilization rates, expressed as the proportion of eggs fertilized ranged 0%-4.58% with a mode of 0%, and were low among the 4 *Acropora* species at CIB (Table 3). These extremely low fertilization rates suggest that gametic incompatibility plays an important role in prezygotic isolation. This result is similar to those in much of the previous literature, which indicates that few species crosses have high rates of fertilization, even between species that potentially can cross often (Table 3). This scenario is further supported by recent experiments on sperm choice and activity, which showed that eggs “prefer” to be fertilized by sperm from the same species (Morita et al. 2006, Willis et al. 2006) and that sperm are preferentially activated by conspecific eggs (Morita et al. 2006, Willis et al. 2006). Second, even though there is a low frequency of interspecific fertilization, breakdown of interspecific embryos after 12 h suggests that either zygotic mortality, i.e., an egg is fertilized but the zygote does not develop, or hybrid inviability, i.e., a hybrid embryo forms but is of reduced viability, continually prevent the development processes from being completed.

Table 3. Summary of interspecific fertilization success of *Acropora* species in the literature

Species	Fertilization success (%)[a]	Species groups[b]	Clades[c]	Locality
A. hyacinthus × *A. cytherea*	50	hyacinthus	C	[f]Magnetic I. (19°15'S, 146°50'E) Palm I. (18°31'S, 146°19'E), Australia
A. pulchra × *A. millepora*	45	aspera	C	
A. selago × *A. millepora*	40	selago/ aspera	C	
A. pulchra × *A. elseyi*	24	aspera/ echinata	C, D	
A. pulchra × *A. cytherea*	10	aspera/ hyacinthus	C	
A. cytherea × *A. selago*	8	hyacinthus/ selago	C	
A. valida × *A. elseyi*	6.4	nasuta/ echinata	A, D	
A. pulchra × *A. hyacinthus*	6.2	aspera/ hyacinthus	C	
A. pulchra × *A. nasuta*	2.5	aspera/ nasuta	C, A	
A. millepora × *A. muricata*	2.2	aspera/ muricata	C, B	
A. millepora × *A. cytherea*	1.7	aspera/ hyacinthus	C	
A. selago × *A. tenuis*	1.4	selago	C	
A. hyacinthus × *A. valida*	1.3	hyacinthus/ nasuta	C, A	
A. millepora × *A. valida*	0.3	aspera/ nasuta	C, A	
A. nasuta-A × *A. nasuta-B*	0.5 ± 1.7[d] 0.0 ± 0.2[e]	nasuta	A	[g]Akajima I., Okinawa, Japan (30°N, 123°E)
A. nasuta-B × *A. nasuta-C*	0.3 ± 0.6[d] 0.1 ± 0.3[e]	nasuta	A	
A. nasuta-A × *A. nasuta-C*	96.8 ± 2.5[d] 1.1 ± 3.1[e]	nasuta	A	
A. muricata-A × *A. muricata-B*	1.2 ± 1.5[d] 1.6 ± 1.5[e]	muricata	B	
A. nasuta × *A. muricata*	0.5 - 75[d] 0.2 - 94.5[e]	nasuta/ muricata	A, B	
A. digitifera × *A. nasuta*	0.2 - 14.3[d] 0 - 3.6[e]	humilis/ nasuta	A	
A. digitifera × *A. muricata*	0[d] 0.7 - 12.6[e]	humilis/ muricata	A, B	
A. intermedia × *A. nasuta*	0	robusta/ nasuta	A, B	
A. intermedia × *A. muricata*	0	robusta/ muricata	B	
A. intermedia × *A. digitifera*	0	robusta/ humilis	B, A	
A. florida × *A. nasuta*	0 - 0.1	florida/ nasuta	C, A	
A. florida × *A. muricata*	0	florida/ muricata	C, A	
A. florida × *A. digitifera*	0 - 1	florida/ humilis	C, A	
A. florida × *A. intermedia*	71.5 ± 28.5[d] 0[e]	florida/ robusta	C, B	
A. tenuis × *A. yongei*	24.2 ± 48.5[d] 46.4 ± 53.5[e]	selago	C	[h]Akajima I., Okinawa, Japan (30°N, 123°E)
A. tenuis × *A. donei*	22.0 ± 37.1[d] 12.9 ± 31.5[e]	selago	C	
A. yongei × *A. donei*	0	selago	C	
A. tenius × *A. verweyi*	0	selago/ verweyi	C, D	
A . tenius × *A. austera*	0	selago/ rudis	C, A	
A. solitaryensis (*AR*) × *A. solitaryensis* (*PL*)	< 1.0			[i]Shirahama (33°43'N, 135°19'E)
A. solitaryensis (*AR*) × *A. pruinosa*	88.0 ± 22.0[d] 48.7 ± 28.9[e]		-	
A. solitaryensis (*PL*) × *A. pruinosa*	0[d] 2.3 ± 3.2[e]		-	
A. muricata × *A. humilis*	0.71 ± 2.27	muricata/ humilis	B, A	[j]Chinwan, Penghu I., Taiwan (23°31'N;119°33'E)
A. muricata × *A. hyacinthus*	0.74 ± 2.40	muricata/ hyacinthus	B, C	
A. muricata × *A. valida*	1.12 ± 5.07	muricata/ nasuta	B, A	
A. humilis × *A. hyacinthus*	1.57 ± 3.69	humilis/ hyacinthus	A, C	
A. humilis × *A. valida*	1.03 ± 3.26	humilis/ nasuta	A	
A. hyacinthus × *A. valida*	2.98 ± 8.32	hyacinthus/ nasuta	C, A	

[a] Data adopted from information summarized in the literature. [b] Species groups defined by Wallace (1999). [c] Phylogenetic clade information summarized by Wallace (1999). [d] Sperm from the former species crossed with eggs of the latter species. [e] Reciprocal crosses. [f] Willis (1997). [g] Hatta et al. (1999). [h] Fukami et al. (2003). [i] Suzuki and Fukami (2011). [j] This study. -, Not available.

Hybridization is not promoted in *Acropora* in marginal coral communities

It was hypothesized that when many congeneric species spawn simultaneously in sympatry, there will be strong selection for efficient gametic recognition, compared to sites where gametes of fewer species spawn in synchrony. This led to the speculation that hybridization may occur more frequently at the periphery of species' ranges (Willis et al. 2006, Richards et al. 2008). However, this scenario was not supported when we compared the *in vitro* interspecific fertilization success rates across geographic regions (Table 3). First, if we take 5% as the "significant" criterion for fertilization success, at the GBR, where *Acropora* diversity is high (76 species) (Wallace 1999), eight of 14 (57.14%) crosses exhibited significant fertilization success (Willis 1997). At Akajima I., Okinawa, Japan, of 35 *Acropora* species recorded, seven of 19 (36.84%) crosses exhibited significant fertilization success (Hatta et al. 1999, Fukami et al. 2003). In contrast, at CIB, Penghu I., Taiwan and Shirahama, Japan, where *Acropora* species diversity is relatively low (Hsieh 2008, Takuma pers. comm.), fertilization success rates were low (with mode of 0%) compared to those of the GBR and Akajima I., except for the cross between *A. pruniosa* and the arborescent morph of *A. solitaryensis* (Suzuki and Fukami 2011). These data reveal an opposite trend to the prediction by Willis et al. (2006) that hybridization will be more frequent at the periphery of a species' range.

Second, not only is species diversity relatively low, but most species in marginal habitats (including CIB) are also distantly related according to morphological phylogenies (Wallace 1999). At the GBR, most instances of high fertilization success are between species pairs from the same clade (clade C) (Table 3). At Akajima I., Okinawa, high fertilization success occurs either by a 1-way cross within the same clade (e.g., *A. nausta-A* × *A. nasuta-C*) or with high variation between clades (e.g., *A. nasuta* × *A. muricata*) (Table 3). At Penghu, the total number of coral species is 75, with only *A. muricata*, *A. humilis*, *A. valida*, and *A. hyacinthus* dominant in shallow waters (Hsieh 2008). These 4 species are classified in 3 clades that had a mode of fertilization successes equal to 0%. Similar species diversities were also reported at other high-latitude coral communities. From the Solitary Is. (> 29°S), a high-latitude coral community in eastern Australia, 14 *Acropora* species were recorded (Wilson and Harrison 2003). From Otsuki, Kochi Prefecture, Japan (> 33°N), 8 *Acropora* species were recorded (Nomura and Takemura 2005, Takemura et al. 2007). *Acropora* species within these assemblages are often distantly related. This differs from the situation in the Caribbean, where co-occurring species are closely related, such as with the *A. cervicornis* group or the *Montastraea annularis* complex. Even though those species are closely related and may occasionally hybridize, mechanisms to prevent hybridization from occurring have also developed in the *M. annularis* complex (Levtian et al. 2004). Future studies of reproductive compatibilities of *Acropora* species are needed in order to confirm that hybridization is less likely to occur in marginal communities of high-latitude regions compared to the GBR and Okinawa, such as at the Solitary Is., Australia and Otsuki, Japan.

In summary, observations of spawning days and times and interspecific fertilization trials of 4 sympatric *Acropora* species were conducted at a non-reefal coral community in CIB, Penghu I., Taiwan. The results showed that *Acropora* spp. in CIB spawned less synchronously compared to previous reports of species in the GBR and Okinawa, and interspecific fertilization was extremely low. Ecological and reproductive analyses indicated that the 4 sympatric *Acropora* species maintain clear species boundaries, and hybridization does not appear to be as frequent at marginal habitats as formerly hypothesized. Thus, the scenario that hybridization facilitates Indo-Pacific *Acropora* species expanding their ranges and adapting to changing environments should be considered with caution.

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