

RESEARCH ARTICLE

Comparisons of population density and genetic diversity in artificial and wild populations of an arborescent coral, *Acropora yongei*: implications for the efficacy of “artificial spawning hotspots”

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We are developing techniques to restore coral populations by enhancing larval supply using “artificial spawning hotspots” that aggregate conspecific adult corals. However, no data were available regarding how natural larval supply from wild coral populations is influenced by fertilization rate and how this is in turn affected by local population density and genetic diversity. Therefore, we assessed population density and genetic diversity of a wild, arborescent coral, *Acropora yongei*, and compared these parameters with those of an artificially established *A. yongei* population in the field. The population density of wild arborescent corals was only 0.27% of that in the artificial population, even in a high-coverage area. Genetic diversity was also low in the wild population compared with the artificial population, and approximately 10% of all wild colonies were clones. Based on these results, the larval supply in the artificial population was estimated to be at least 1,400 times higher than that in wild *A. yongei* populations for the same area of adult population.

Key words: artificial larval source, coral restoration, direct larval seeding, larval supply, microsatellite

Implications for Practice

- To enhance larval supply, it is important to enhance both population density and genetic diversity of adult corals when creating “artificial spawning hotspots.”
- Theoretically, even a small (e.g. 100 m²) “artificial spawning hotspot” can supply enough coral larvae to restore a large area (over 10 ha) of arborescent *Acropora* corals.
- Spawn from 15 genotypes was sufficient to produce genetic diversity comparable to wild populations of *Acropora yongei*.

Introduction

Due to global stressors and local anthropogenic disturbances, coral reefs are declining worldwide in both coverage (Nystrom et al. 2000; Gardner et al. 2003; De'ath et al. 2012) and species diversity (Carpenter et al. 2008). In 2016, the northern Great Barrier Reef lost 67% of its corals (Hughes et al. 2018). That same year, Sekisei Lagoon, in southern Okinawa, Japan's largest coral reef, also suffered long-term bleaching of 90% of its corals and 70% coral mortality (Nakamura 2017). Following effective management of local anthropogenic impacts, a range of local interventions to reduce pressures and encourage corals such as controlling crown-of-thorns seastars, coral transplantation, and propagation may be required.

So far, coral restoration techniques have been applied all over the world (Shafir et al. 2006; Zimmer 2006; Edwards 2010; Barton et al. 2015; Bayraktarov et al. 2016; Lirman &

Schopmeyer 2016; Omori et al. 2016). Utilizing reproductive characters of scleractinian corals (reviewed by Richmond & Hunter 1990; Harrison 2011), new colonies have been produced either asexually (fragments) or sexually (crossing gametes and collecting larvae) for reef restoration (Linden & Rinkevich 2011). Recently, coral gardening by transplanting fragments was employed and achieved restoration of about 3 ha in a reef in Okinawa Prefecture (Omori et al. 2016). However, transplantation is quantitatively limited, labor-intensive, and costly, and what is more, a single local disturbance such as bleaching, a crown-of-thorns seastar outbreak, or a severe typhoon can easily nullify such efforts. Therefore, we focused on creating a stable and persistent supply using sexually produced coral larvae.

There have been some attempts at direct larval seeding for reef restoration. Early studies in Western Australia (Heyward

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et al. 2002) and Okinawa (Omori et al. 2003) harvested spawn slicks and used competent coral larvae in seeding experiments. Subsequent experimental scale reseeding efforts in Palau (Edwards et al. 2015) and the Philippines (de la Cruz & Harrison 2017) using larvae sourced from captive spawners rather than slicks demonstrated that initial recruitment can be enhanced. In addition, feasibility of reef restoration based upon larval supply has also been discussed, and different types of artificial larval sources have been suggested (Amar & Rinkevich 2007; Horoszowski-Fridman et al. 2011; Rinkevich 2014; Montoya-Maya et al. 2016). However, there have been few reports on the effectiveness of direct larval seeding in relation to the ecology of target coral species.

In this study, the target coral was an arborescent *Acropora* that is relatively important for its contributions to fish habitats (Nanami et al. 2013). The genus *Acropora*, which is one of the dominant genera in Indo-Pacific shallow-water coral reefs, is a broadcast spawner and a simultaneous hermaphrodite. Some *Acropora* species having arborescent or bottlebrush colonies reproduce not only sexually (i.e. spawning) but also asexually (i.e. fragmentation); however, their colony-level fecundity is low (Wallace 1986). Indeed, comparing the amount of eggs per colony weight, arborescent, and bottlebrush species spawned approximately one-third that of tabular and corymbose species (Suzuki, unpublished data). Hence, when an arborescent or bottlebrush *Acropora* population is destroyed by large disturbances such as mass-bleaching events leading to high levels of mortality, recovery is slow, due to minimal larval supply. In addition, recent population genetics studies have shown that the dispersal distance of acroporid corals is much shorter (less than a few tens of kilometer) than previously thought (reviewed by Van Oppen & Gates 2006; Shinzato et al. 2015; Zayasu et al. 2016).

Furthermore, although *Acropora* does not cross with clonal colonies or self-fertilize (Heyward & Babcock 1986; Isomura et al. 2013), levels of clonality within populations of arborescent species may be higher than those of tabular or corymbose species because of their propensity for fragmentation. Based on these ecological features, we considered arborescent *Acropora* an optimal target for development of restoration methods aimed at artificially enhancing larval supply. In this context, pre-existing densities and genetic diversities of populations are essential to assess the success of coral recruitment. To enhance reproduction of arborescent *Acropora*, we tried to optimize density to achieve a very high level of fertilization by creating “artificial spawning hotspot.” The present study demonstrates that such hotspots can generate higher larval supply than wild populations. That is, the higher density of the artificially created population without clonal colonies leads to higher levels of cross-fertilization at spawning time because self-fertilization of *Acropora* corals is quite rare (Heyward & Babcock 1986); hence, a given number of sexually produced colonies produce more embryos and subsequently more larvae.

To estimate the larval supply of a spawning hotspot, we determined the population density and genetic diversity of the pre-existing wild population because spatial distribution and cross compatibility of adult colonies determine reproductive success (i.e. larval supply). The target taxon in this study was an arborescent species, *Acropora yongei* Veron and Wallace 1984, which can dominate inner reef and lagoon areas of Indo-Pacific coral reefs. We compared population densities and genetic diversities of *A. yongei* populations between wild and artificial populations. For an artificial spawning hotspot, we used 4-year-old corals from which gametes were collected, fertilized, and settled on grid plates in 2011 (Fig. 1) (Suzuki et al.



Figure 1. *Acropora yongei* assemblages at an artificial spawning hotspot at Sekisei Lagoon, Ishigaki Island, Okinawa, Japan.

2013). We tested two hypotheses that sexual reproduction (i.e. larval seeding) can be used to create high-density and genetically diverse populations, and that larval supply in the artificial spawning hotspot can exceed that of wild populations, particularly for arborescent *Acropora* corals.

Methods

Comparison of Population Density and Sample Collection for Genetic Analysis of Arborescent *Acropora*

Two wild populations of the target species, *Acropora yongei*, were selected in Sekisei Lagoon, located in the southern Ryukyu Archipelago, Japan, one with high coral coverage and the other with low coverage. At both the high- and low-coverage sites, two coral colonies were randomly selected to represent the center points of two subsites (Fig. 2). Then, conspecific corals were identified within a 10-m radius from the central colony at the high-coverage site, and within a 40-m radius at the low-coverage site. Direction and distance from the central colonies were recorded for each conspecific colony. For the artificial population, the number of *A. yongei* colonies was counted on 36 plates (each 1 m²), and an average density was calculated. These artificial plates were established by settling sexually reproduced *A. yongei* larvae on grid-like fiberglass-reinforced plastic plates, elevated 50 cm above the seafloor on supporting steel rods (Suzuki et al. 2011, 2013) (Fig. 1). These plates were installed at six depths from 5 to 18 m in the low-coverage site of Sekisei Lagoon in 2011 (Suzuki et al. 2013). At that time, *A. yongei* larvae were produced from 15 wild adults randomly collected near these study sites and seeded on plates. Approximately 2-cm branch fragments were collected from all colonies of the wild populations at both sites (24 colonies in total) in April 2016 and 18 artificial colonies (3 colonies in each depth) in April 2015. Coral fragments were preserved in 99% ethanol and brought to the laboratory for analyses. All collections were approved under permit from the Okinawa Prefectural Government.

DNA Extraction and Genotyping

Genomic DNA was extracted using Maxwell RSC Blood DNA Kits (Promega KK, Tokyo, Japan) with the Promega

Maxwell RSC System AS4500 (Promega KK, Tokyo, Japan) and quantified using a NanoDrop™ 1,000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). In all, 13 microsatellite loci from published data (Shinzato et al. 2014) with polymorphic characteristics were adopted for genotyping coral colonies. All PCR amplifications were carried out in a 10 µL reaction volume containing forward primer with M13 tail at the 5'-end (1 µL), reverse primer (0.5 µL), universal 6-FAM fluorescence-labeled M13 primer (0.5 µL), 10 ng/µL template DNA, AmpliTaq Gold 360 Master Mix (Qiagen, Hilden, Germany), and MilliQ water (Merck Millipore, Darmstadt, Germany). The following PCR protocol was adopted: 10 minutes at 95°C, followed by 32 cycles each of 30 seconds at 95°C, 30 seconds at 52°C, and 30 seconds at 72°C, with an extension of 1 minute at 72°C in the final cycle. Fragment sizes were determined using GeneMapper software version 5.0 (Thermo Fisher Scientific) by comparison with a GeneScan™ 500 LIZ (Thermo Fisher Scientific) internal lane size standard, on Applied Biosystems 3730xl Genetic Analyzers (Thermo Fisher Scientific).

Identification of Clonal Multilocus Genotypes and Genotypic Diversity

To estimate the rate of clonal colonies in the populations, we first checked for the presence of clonal multilocus genotypes (MLGs). In each population, we calculated clonal richness, $R = (N_g - 1) / (N_r - 1)$ (Dorken & Eckert 2001), where N_g indicates the number of MLGs and N_r indicates the number of colonies genotyped. We also calculated the probability p_{SEX} , with the program GENCLONE version 2.0 (Arnaud-Haond & Belkhir 2007) to test whether identical replicates are truly clonal individuals. Genepop 4.2 (Raymond & Rousset 1995) was used to calculate a deviation index from Hardy–Weinberg equilibrium (HWE) for each population using the Markov chain method with 1,000 batches and 10,000 iterations per batch, and also to test for deviation from expected linkage equilibrium. The R package, poppr v2.4.1 (Kamvar et al. 2014), was used to assess genotypic diversity, $D = \lambda \times N / (N - 1)$, which was the complement of Simpson's diversity index (λ is the probability that two individuals randomly selected have different MLGs) for sample size (N). To test for the presence of null alleles, the

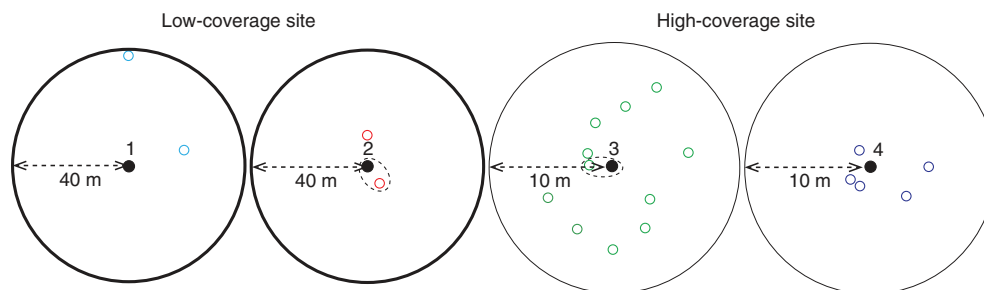


Figure 2. Overview of sample collection. Black circles with numbers indicate central colonies. Two central colonies were selected in each of two habitats, one with low coral coverage and one with high coverage. Colored circles indicate spatial distributions of peripheral colonies. Dashed circles indicate a coral colony and its clonal lineage.

program Micro-Checker (confidence interval for Monte Carlo simulations 99%) was used.

Measures of Genetic Diversity Without Clones

To assess the genetic diversity of both wild and artificial populations without clone colonies, the number of alleles per locus was calculated using FSTAT 2.9.3.2 (Goudet 1995). Allelic richness (A_r), observed heterozygosity (H_o), expected heterozygosity (H_e), the p -value of the goodness of fit to HWE expectations test using Fisher's method (HWE), and inbreeding coefficients (F_{IS}) were also calculated using the R package, *diveRsity* (Keenan et al. 2013) with 1,000 bootstrap replicates. We estimated Nei's gene diversity (Nei 1973) or of the average expected heterozygosity over all loci, which is a frequently used estimate of the extent of genetic variability in the population. Statistical tests were performed using R version 3.2.4.

Results

Comparison of Population Density

Wild Populations. At the low-coverage site, only two *Acropora yongei* coral colonies were found within 40-m radii around each central coral (population density was 0.0006 colonies/m²). Average distances between corals were 20.7 ± 5.2 m (average \pm standard error) at this site. At the high-coverage site, 11 and 5 *A. yongei* were found within 10-m radii from each central coral (population density was 0.029 colonies/m²). Average distances between corals were 3.6 ± 0.3 m at this site.

Artificial Populations. The average population density was 8.2 colonies/m² at 5 m depth, 10.8 at 6 m, 9.3 at 9 m, 12.5 at 12 m, 12.0 at 15 m, and 10.7 at 18 m. The average population density at all depths was 10.6 ± 0.8 colonies/m².

Comparison of Genetic Diversity

All loci that we employed were polymorphic at Sekisei Lagoon, except locus 11292m4 in the wild (Table 1). We detected a

total of 67 alleles at 13 loci among 42 individual *A. yongei*. The number of alleles observed at each locus ranged from 2 (11292m4, 11543m5, and 12130m5) to 10 (11745m3). Deviation from HWE was not detected after the sequential Bonferroni procedure (Rice 1989). The presence of null alleles was suggested at 7961m4 in the wild, but all loci were retained for further analyses because these trends were not seen in artificial populations.

Clonal richness (R) was 1.000 in the artificial population and 0.916 in the wild populations. Genotypic diversities (D) were 1.000 and 0.993 in artificial and wild populations, respectively. No clonal lineage was detected in any artificial population. Two clonal lineages were found among wild populations (Fig. 1). There were no clonal colonies in which ramets appeared to have the same genotype by chance, or that appeared as genets, due to scoring errors or to somatic mutations ($p_{SEX} < 0.01$). Duplicate data from the two wild clonal lineages were excluded from subsequent analyses (artificial, $N = 18$; wild, $N = 22$).

Inbreeding coefficients (F_{IS}) were 0.024 and 0.064 in artificial and wild populations, respectively (Fig. 3). The F_{IS} estimated in wild populations was not significantly greater than

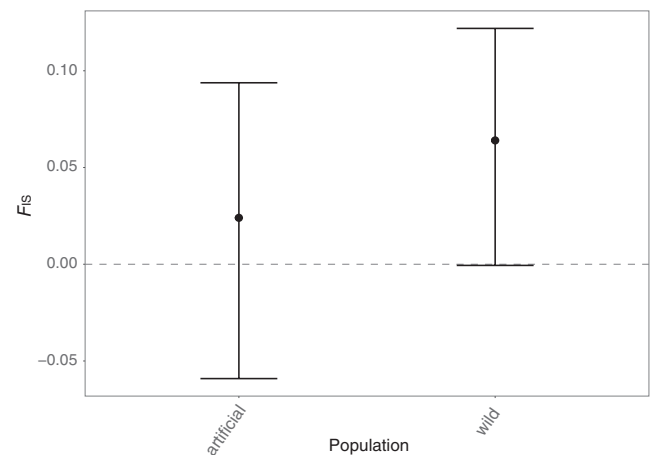


Figure 3. Inbreeding coefficients (F_{IS}) did not differ significantly between artificial and wild *Acropora yongei* populations. Black circles are estimated F_{IS} values, and the error bars show $\pm 95\%$ CI.

Table 1. Number of alleles per locus, allelic richness (A_r), observed (H_o), and expected heterozygosity (H_e) for each locus and population.

		8346m3	7961m4	11745m3	12406m3	11543m5	530m4	11401m4	441m6	11292m4	7203m5	12130m5	8499m4	10366m5	Overall
Artificial	Number of alleles	5	3	6	6	2	2	6	4	2	8	2	5	5	56
$N = 18$	Allelic richness	4.83	3	5.63	5.56	1.99	2	5.73	3.94	2	6.62	1.96	4.26	4.82	4.03
	H_o	0.67	0.44	0.94	0.78	0.22	0.28	0.78	0.56	0.28	0.44	0.17	0.56	0.61	0.52
	H_e	0.73	0.55	0.79	0.7	0.28	0.31	0.75	0.67	0.24	0.63	0.15	0.51	0.58	0.53
Wild	Number of alleles	6	5	10	7	2	5	7	5	1	7	2	5	5	67
$N = 24$	Allelic richness	5.14	4.55	8.63	5.94	1.99	4.32	6.39	4.38	1	5.73	2	4.06	4.8	4.53
	H_o	0.68	0.41	0.73	0.86	0.23	0.45	0.86	0.59	0	0.45	0.27	0.36	0.77	0.51
	H_e	0.73	0.76	0.84	0.76	0.2	0.61	0.74	0.63	0	0.56	0.24	0.42	0.72	0.55

that estimated in artificial populations ($\pm 95\%$ CI). Moreover, the F_{IS} value in both wild and artificial populations did not differ significantly from zero ($\pm 95\%$ CI).

Estimates of average expected heterozygosity (H_e) over all loci under a HWE did not differ significantly between them either (Student's t test: $p > 0.05$, $t = -0.2601$, $df = 24$) with values of 0.530 ± 0.060 CI and 0.554 ± 0.072 CI, respectively. In the present study, there was no reduction of genetic diversity in artificial populations of *A. yongei*. In other words, crossing 15 colonies produced genetic diversity comparable to that of wild populations for this species in Sekisei Lagoon.

Discussion

The population density of wild *Acropora yongei* was 0.0006–0.029 colonies/m² in this study area in the low- and high-density populations, respectively. The density in artificial spawning hotspots was 10.6 colonies/m², more than 365 times higher than in wild populations. The average linear distances between corals were 3.6 m in the high-coverage site and 20.7 m in the low-coverage site. Fertilizable distances of marine sessile organisms differ depending on species: 50 cm in ascidians (Grosberg 1991) and 10 m in the scleractinian coral, *Seriatopora hystrix* (Warner et al. 2016). Because all *Acropora* species are broadcast spawners, which make bundles of eggs and sperm to carry gametes to the surface, the fertilizable distance may be longer than for other species. However, a sperm concentration of 10^6 – 10^7 /mL was required to achieve a high fertilization rate ($>80\%$) based on laboratory experiments (Omori et al. 2001; Nozawa et al. 2015). Moreover, the expected fertilizable distance is less than 2 m even in *Acropora* corals, because the sperm concentration rapidly dilutes after the bundle is broken (Iwao et al., unpublished data; Okinawa Prefecture Government 2017). This was estimated by numerical simulation using a sperm diffusion coefficient, and the fertilizable area ($>10^5$ /mL sperm concentration area) could be kept more than 20 minutes when nine colonies distributed at 2-m intervals spawned simultaneously. Of course, bundles could disperse more than 2 m from the parental colony. However, most bundles were broken within a few minutes after rising to the surface in open water (G. Suzuki 2017, Ishigaki, Japan, personal observation), although they can maintain their form over 30 minutes in aquarium. That is, this mass spawning system of *Acropora* corals could ensure reproductive success under high population density conditions. Considering these results, wild *A. yongei* corals at the low-coverage site may have a low probability of fertilization.

In addition, genetic analyses detected clonal colonies from wild populations within 7 m of one another at the low-density site and 1.7 m at the high-density site. Although clonal richness was low, this suggests that asexual reproduction in *A. yongei* in study area occurs naturally via fragmentation due to physical disturbances caused by typhoons or wave action. Considering that the congeneric *Acropora tenuis* does not appear to propagate by fragmentation in this study area (Zayasu et al. 2016), this may reflect the structure of arborescent species, such as skeletal strength, and the rate of fragment reattachment.

Estimated F_{IS} in both wild and artificial populations was as expected under HWE, even though the artificial population represented fertilization with gametes from 15 colonies. This suggests that 15 colonies are sufficient to produce adequate genetic diversity, at least for the next generation.

Previously, there have been assertions that arborescent *Acropora* corals reproduce using many asexual recruits derived from fragmentation (Fong & Lirman 1995; Nakamura & Nakamori 2006). However, this study showed that 90% of wild arborescent corals in the study site were sexually recruited. Also in the Atlantic, the threatened coral species *Acropora cervicornis* has been thought to rely on asexual fragmentation, but experiments have shown that fragmentation may not be a significant source of recruits (Vollmer & Palumbi 2007; Mercado-Molina et al. 2014). Clearly, larval recruitment by sexual reproduction is important to maintain population densities even in arborescent corals. In addition, sexually produced seedlings could enhance local genetic variability within a short period (Baird et al. 2009).

Based on these results, we estimated the larval supply capability of both wild and artificial *A. yongei* populations. In these wild populations, an area of more than 365 m² is required so as to permit cross-fertilization of 10 colonies, while only 1 m² is necessary in the artificial population. In *Acropora* corals, crosses between gametes from six or more colonies provide the highest fertilization rate (Iwao et al. 2014). The fertilization rate is decreased in wild populations mainly by sperm dilution ($<50\%$, G. Suzuki, unpublished data) and by incompatibility between clonal colonies, while it can be kept at high levels in artificial populations. In addition, a larval collecting device called a “larval cradle” (Okada et al., unpublished data) can be used in the artificial population, which allows us to achieve nearly 100% fertilization in open water by enclosing gametes within a cylindrical plankton net. Moreover, larval survival during the swimming stage is very low in wild populations due to predation (approximately 50% within 10 days after spawning) (Connolly & Baird 2010), whereas a larval cradle can maintain more than 90% survival in artificial populations. Based on these estimates, the larval supply capability per unit area of artificial population is at least 1,400 times higher than that of wild populations, even at high-coverage sites. We can design very compact artificial populations with even higher fertilization capacity by optimally arranging sexually produced seedlings. Such artificial spawning hotspots can potentially supply massive numbers of coral larvae sustainably with little or no maintenance cost. For example, 100 m² of the hotspot could supply as many larvae as those from the wild population of over 14 ha annually. That is, it would be easier to establish 100 m² of artificial hotspot only once than to transplant the large number of fragments to several hectares of damaged reef every year.

Natural larval recruitment of *Acropora* corals occasionally reaches 1,000–10,000 settlers/m² (i.e. coral spats on settlement plates retrieved approximately 2 weeks to 1 month after mass spawning) in Indo-Pacific reefs, such as Okinawa (Suzuki et al. 2012) and the Great Barrier Reef in Australia (Hughes et al. 1999; Edmunds et al. 2015). However, these are cases focusing only on outer reef edges and slopes. In inner bays and

lagoons, where arborescent corals are dominant, often 100 or fewer settlers/m² are encountered (Suzuki et al. 2012). This is probably caused by poor larval supply of arborescent *Acropora* corals; therefore, artificial spawning hotspots established in inner bays and lagoons could potentially contribute significantly to coral regeneration efforts after disturbances such as bleaching events or crown-of-thorns seastar outbreaks. Of course, these artificial spawning hotspots could also be damaged by these disturbances; some contrivances in installation method and structure design to avoid these effects would be required. These technological developments are future tasks to be solved as soon as possible.

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