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RESEARCH ARTICLE

Genetic diversity of farmed and wild populations of the reef-building coral, *Acropora tenuis*

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Transplantation of nursery-farmed corals is a primary management tool for restoration of degraded coral reefs. However, there have been concerns about the potential loss of genetic diversity in nurseries due to asexual propagation methods used to prepare transplants. Two coral nurseries at Maeganeku and Onna, Okinawa Island, Japan, furnish source material for regional restoration activities. Using 13 microsatellite markers, this study compared the genetic diversity of 132 *Acropora tenuis* colonies from these nurseries with that of 298 wild colonies from 15 sites across the Nansei Islands. Even though no clonal colonies were detected at wild sites, we estimated clonal richness of farmed corals to be 0.523 (Maeganeku) and 0.579 (Onna). Genotypic diversity is high in the nursery populations, 0.894 (Maeganeku) and 0.937 (Onna), but lower than in the natural populations (1.000). However, expected heterozygosity did not differ significantly between locations, including the coral nurseries (one-way analysis of variance, $p > 0.05$). Inbreeding coefficients of nursery populations (Onna, −0.019 to Maeganeku, 0.097) fell within the range of estimates from wild populations (Sesoko, −0.058 to Maeda, 0.278). Furthermore, based on Structure analysis, farmed *A. tenuis* comprises the same genetic population that occupies the surrounding natural area. Thus, given no additional statistically significant increase of clonal colonies within nurseries, using farmed coral assemblages for reef restoration may be preferable to transplanting and damaging wild assemblages. Coral gametes of farmed colonies may also be used to produce coral larvae for transplantation of sexually propagated corals.

Key words: coral reefs, microsatellite, outplanting nursery corals, population genetics, reef restoration, transplantation

Implications for Practice

- To avoid reduced genetic diversity in coral nurseries, coral genotypes should be determined and records of asexual fragmentation should be kept. Theoretically, these could be useful for selective breeding of strains resilient to various impacts.
- Restoration efforts using sexually produced coral colonies should be increased whereas use of asexually produced coral fragments needs careful attention, because most colonies of *Acropora tenuis* do not propagate asexually in the wild.

Introduction

Coral reefs are among the most diverse ecosystems on Earth (Wilkinson 2002). Corals provide valuable ecosystem services, including food and habitat for other marine species (Spalding et al. 2001). However, coral reefs have been disappearing due to both natural and anthropogenic disturbances (De'ath et al. 2012; Hughes et al. 2017). From 1998 to 2008, 19% of the world's coral reef coverage was lost (Wilkinson 2008). Given current climatic conditions, the great majority of coral reefs globally will experience severe bleaching at least twice per decade by 2040 (Heron et al. 2017).

In the past decade, coral transplantation has been recognized as a prime management tool for restoration (Epstein et al. 2003; Rinkevich 2008). Source materials for transplantation are obtained by both asexual reproduction (coral fragments) and sexual reproduction (in situ or ex situ settled planula larvae) (Nakamura et al. 2011; Guest et al. 2014). However, transplantation of asexually propagated corals has raised concerns about reduced genetic diversity (Rinkevich 1995; Shearer et al. 2009). Although asexually propagated corals increase the number of colonies, these new colonies are genetically identical to their parents (i.e. clones or ramets) (Harper 1977; Hughes & Jackson 1985). Because self-fertilization is rare in most *Acropora* (Willis et al. 1997; van Oppen et al. 2002), reduced genetic diversity of coral nurseries might harm future fertilization due to inbreeding depression and gamete self-incompatibility.

In Okinawa, both commercial and governmental projects for coral transplantation have been conducted. In the summer of

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Figure 1. Coral nurseries; Maeganeku Farm in Onna Village, Okinawa, Japan. Coral colonies are maintained on iron posts.

1998, due to a mass bleaching event, average coral cover at several sites around Okinawa Island decreased to 49% of 1995 levels (Hongo & Yamano 2013). In order to produce coral colonies by asexual reproduction for transplantation, the local fishermen of the Onna Village Fisheries Cooperative collected coral fragments (about 5 cm in length) four times, in 1998, 1999, 2003, and 2004 under permits from Okinawa Prefecture. Corals were reared in two donor coral nurseries, Onna Farm (approximately 26.51°N, 127.85°E) and Maeganeku Farm (approximately 26.44°N, 127.79°E) (Higa & Omori 2014) (Fig. 1). Coral fragments of 54 species were collected locally to create the nurseries (Higa et al. 2017).

These donor colonies were used to produce coral fragments for transplantation as part of Okinawa Prefecture's 7-year "Coral Reef Preservation and Rehabilitation Project," started in 2011 (Omori et al. 2016). More than six acroporid species, including *Acropora tenuis* (Dana, 1846), were transplanted into an area of 2.1 ha (Higa et al. 2017). Although there have been concerns about possible loss of genetic diversity within nurseries, no cross-species genetic markers for acroporid corals were available until Shinzato et al. (2014).

Acropora tenuis has been used for reef restoration projects around the globe (Omori et al. 2008; Petersen et al. 2008; Baria et al. 2010; Rocker & Brandl 2015). We compared genetic diversity of farmed and wild populations in Okinawa, Japan. This study also sought to determine the degree of clonality and the genetic population structure in both farmed and wild populations.

Methods

Collection of Samples

Samples of *Acropora tenuis* were collected from coral colonies in 2 coral nurseries (Onna and Maeganeku) and at 15 wild sites in the Nansei Islands, Japan (Fig. 2). For wild locations, 15 sites that had 10 or more colonies of *A. tenuis* within approximately 3 ha were sampled at depths less than 10 m (Zayasu et al. 2016). For both wild and farmed samples, a branch fragment approximately 2 cm was collected from each colony and preserved in 99% ethanol. For farmed corals, each source colony was identified with a numbered plastic tag to track genotype.

DNA Extraction, PCR, Sequencing Procedures

Genomic DNA was extracted from coral fragments using DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany). To aid digestion, coral fragments were cut into small pieces using a nipper. After DNA extraction, total DNA concentration was determined using a NanoDrop1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.). We selected 13 microsatellites from universal primer sets for the genus *Acropora* (Shinzato et al. 2014). Fluorescent primer labels (6-FAM, CAG, and T7 terminator), polymerase chain reaction (PCR) conditions, and sequencing were as described previously (Zayasu et al. 2016).

Genotyping Statistical Analysis

To identify microsatellite loci that may be under selection, the program BayeScan v2.1 (Foll & Gaggiotti 2008) was used to locate truly adaptive regions of the genome. Loci that demonstrated significantly higher or lower among-population genetic differentiation than expected under neutrality were identified as outliers.

Fragment sizes were determined by comparing fragments against an internal standard using GeneMapper software version 5.0 (Thermo Fisher Scientific) with a GeneScan500 LIZ

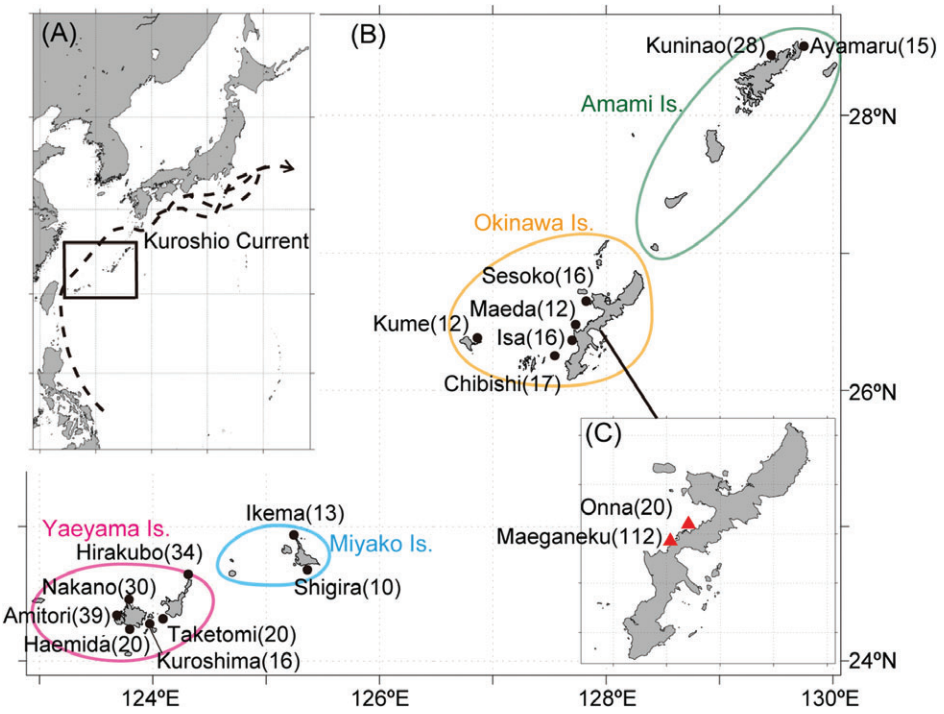

Figure 2. Map of the study area. (A) A map of East Asia, including the study area (solid square) and the Kuroshio Current (dashed arrow). (B) The Nansei Islands, Japan. Colored circles represent island groups: Amami Islands, green; Okinawa Islands, orange; Miyako Islands, cyan; Yaeyama Islands, magenta. Black circles with names show sampling locations, and numbers in parentheses show the number of colonies collected. (C) Okinawa Main Island. Red triangles with names represent coral nurseries and numbers in parentheses show the number of colonies collected.

(Thermo Fisher Scientific). When more than two samples had identical genotypes, we treated them as ramets (clonal replicates). To justify truly clonal individuals, we used GenClone v2.0 (Arnaud-Haond & Belkhir 2007) to calculate p_{sex} values for each multilocus genotype, and significance determined at $p_{sex} < 0.05$. Two frequently used measures were calculated: clonal richness, R, with the modification of Dorken and Eckert (2001) [$R = (Ng - 1)/(Nr - 1)$]; and D, the complement of Simpson's diversity index (genotypic diversity; λ). D, corrected for finite sample size [$\lambda \times N/(N - 1)$], was calculated using the R package, poppr v2.4.1 (Kamvar et al. 2014).

The number of alleles per locus (allelic richness, Ar) was calculated using FSTAT 2.9.3 software (Goudet 1995). Observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were calculated using GenAlEx, version 6.5 (Peakall & Smouse 2012). Pairwise population F_{ST} values were estimated using both GenAlEx 6.5 and Arlequin 3.5.2.2 (Excoffier & Lischer 2010). The exact p-value for Hardy–Weinberg equilibrium was calculated with the Markov chain method using Genepop software v4.2 (Raymond & Rousset 1995) for each locus in each population. The number of unique alleles in a population, private allelic richness, was estimated with HP-Rare 1.1, a program that performs rarefaction to correct for differences in sample size (Kalinowski 2005). Statistical analyses were carried out using MATLAB R2016a (MathWorks, Natick, MA, U.S.A.). Inbreeding coefficient (F_{IS}) values and 95% confidence intervals were calculated using the diveRsity package of R (Keenan et al. 2013), using default parameters setting and 999 bootstraps.

Population genetic structure was examined across neutral nine loci using Bayesian methods in Structure (Pritchard et al. 2000) with no a priori information regarding sampling locations. We then used Structure Harvester (Earl & Vonholdt 2012), according to the method of Evanno et al. (2005) to infer the optimal number of genetic clusters (K value). We used CLUMPP (Jakobsson & Rosenberg 2007) to combine the outputs of 20 iterations from Structure at the most appropriate K value.

Results

Overview

Among wild and farmed populations, we detected a total of 163 alleles, and the number of observed alleles per locus ranged from 6 (441m6) to 22 (11745m3). BayeScan analysis identified four loci (8346m3, 11745m3, 530m4, and 4546m2) as significant outliers (Fig. 3). We used potential outliers as well as neutral loci to detect clonal colonies, because inclusion of markers under selection allows greater understanding of population genetic structure in nonmodel organisms (Batista et al. 2016). However, these four outlier loci were eliminated from all other analyses due to expectations of neutrality.

Clonal Structure

In all, 298 colonies from 15 wild sites (Zayasu et al. 2016) and 132 colonies from 2 coral nurseries were investigated

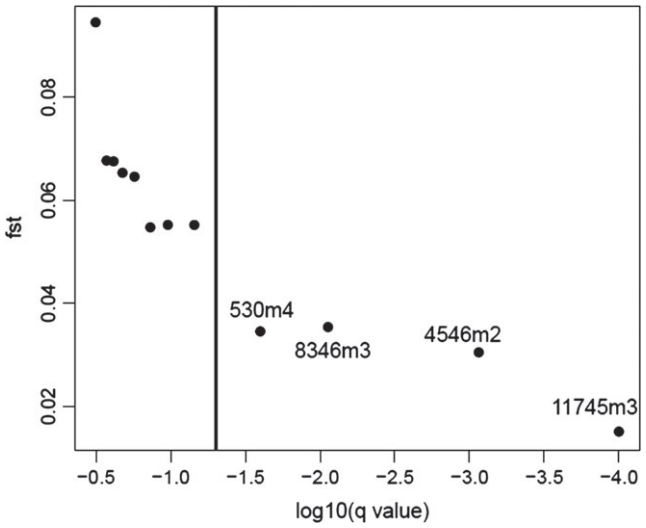

Figure 3. F_{ST} plotted versus $\log_{10}$ (q-value), which is the false discovery rate (FDR) analog of the p-value. A vertical line represents the threshold (FDR = 0.05) and dots with the names of markers indicate outlier loci, which may be affected by selection.

Table 1. Clonal richness among 430 wild and farmed *Acropora tenuis* colonies obtained by genotyping 13 microsatellite loci. Nr, number of samples; Ng, the number of multilocus genotypes; $R = (Ng - 1)/(Nr - 1)$; D, the complement of Simpson's index (genotypic diversity).

		Nr	*Ng*	R	D
Ayamaru	Wild	15	15	1.000	1.000
Kuninao	Wild	28	28	1.000	1.000
Sesoko	Wild	16	16	1.000	1.000
Maeda	Wild	12	12	1.000	1.000
Isa	Wild	16	16	1.000	1.000
Chibishi	Wild	17	17	1.000	1.000
Kume	Wild	12	12	1.000	1.000
Ikema	Wild	13	13	1.000	1.000
Shigira	Wild	10	10	1.000	1.000
Hirakubo	Wild	34	34	1.000	1.000
Nakano	Wild	30	30	1.000	1.000
Haemida	Wild	20	20	1.000	1.000
Taketomi	Wild	20	20	1.000	1.000
Kuroshirna	Wild	16	16	1.000	1.000
Amitori	Wild	39	39	1.000	1.000
Maeganeku	Farmed	112	59	0.523	0.894
Onna	Farmed	20	12	0.579	0.937
	Total	430	369	0.858	0.993

(total = 430). All 13 microsatellite loci encoded at sampled sites were polymorphic. Multilocus genotypes were determined from all 132 nursery colonies.

No clonal colonies were detected among the 298 wild colonies (Zayasu et al. 2016). However, several asexually produced colonies (ramets) from coral nurseries had the same multilocus genotypes, as did 59 genetically distinct individuals (genets) from 112 samples at the Maeganeku Farm and 15 genets from 20 samples at the Onna Farm as a result of artificial fragmentation (Table S1). For all these ramets, the probability of clonal identity was estimated using GenClone software. All p_{sex} values were less than 0.05 for each clone, meaning that there were no clonal colonies in which ramets appeared to have the same genotype by chance, or that appeared as genets due to somatic mutations or scoring errors. Clonal richness (R) and genotypic diversity (D) revealed the same trend. Clonal richness ranged from 0.523 to 0.579 (Table 1). Genotypic diversities in wild populations were 1.000 in all wild populations, and 0.894 at the Maeganeku Farm and 0.937 at the Onna Farm. Clonal ramets were removed from further analyses, and the remaining 369 genets were used for subsequent analyses.

Genetic Diversity

Nine neutral microsatellite loci were used for subsequent analyses. In wild populations, expected heterozygosity (*He*) ranged from 0.484 ± 0.067 (Kuroshima) to 0.693 ± 0.034 (Kuninao), while nursery *He* was 0.545 ± 0.053 at Onna Farm and 0.642 ± 0.041 at Maeganeku Farm (Table 2). *He* did not differ significantly between locations, including the coral nurseries (one-way analysis of variance [ANOVA], $p > 0.05$), indicating that there has been no detectable loss of genetic diversity in the coral nurseries. Private allelic richness is also important as an index of genetic distinctiveness. Notably, Maeganeku Farm had the highest private allelic richness (0.61) indicating that local distinctiveness was retained (Table 3).

Among both wild and farmed populations, inbreeding coefficients (F_{IS}) of several loci were significant after removal of replicated genotypes (Table 2). F_{IS} differed significantly between Ayamaru and Maeda, Sesoko and Maeda, Sesoko and Iha, Onna and Maeda, Maeda and Ikema, Maeda and Shigira, and Maeda and Amitori. In other words, the inbreeding levels at both coral farms were within the range of variation observed in wild populations (Fig. 4).

Population Differentiation

Population differentiation, as indicated by GenAlEx and Arlequin, showed the same tendency. Genetic variation among sampling sites is moderate (3% of total), but significant (AMOVA [analysis of molecular variance], $p < 0.01$). The greatest genetic distance (0.127) was found between Onna and Kuroshima (Table S2). Based on the optimal number of populations inferred by Structure Harvester, there were two populations in the study area: one includes study sites from the northern Nansei Islands and the coral nurseries (red color), and the other includes the southern Nansei Islands (green color) (Fig. 5).

Discussion

Rehabilitation of coral reefs by means of sexual propagation is labor-intensive and expensive (Omori 2011), compared to asexual propagation. However, restoration efforts using sexually propagated corals should be increased, because most colonies of *Acropora tenuis* do not propagate asexually in local wild populations (Zayasu et al. 2016). Among *A. tenuis* colonies in coral

Table 2. Number of alleles, allelic richness (Ar), observed heterozygosity (*Ho*), expected (*He*) heterozygosity, and inbreeding coefficients (F_{IS}) for each locus and location using nine neutral microsatellite loci. An asterisked F_{IS} indicates a significant deviation from Hardy–Weinberg equilibrium ($p < 0.05$) after sequential Bonferroni correction (Rice 1989).

	Locus	*7961m4*	*12406m3*	*11543m5*	*11401m4*	*441m6*	*11292m4*	*8499m4*	*7203m5*	*12130m5*
Maeganeku, cultured, $N = 112$	Number of alleles	3	12	7	10	4	10	7	6	4
	Ar	2.959	8.115	4.641	5.489	3.733	4.255	3.652	4.141	2.741
	Ho	0.153	0.814	0.593	0.559	0.814	0.271	0.729	0.627	0.661
	He	0.571	0.865	0.712	0.777	0.670	0.589	0.532	0.582	0.480
	F_{IS}	0.733*	0.059	0.166*	0.280*	−0.214	0.540*	−0.369	−0.077	−0.377
Onna, cultured, $N = 20$	Number of alleles	3	5	2	6	4	5	3	3	3
	Ar	2.833	4.81	2	5.953	3.812	4.829	2.976	2.833	3
	Ho	0.083	1.000	0.333	0.750	0.583	0.667	0.417	0.500	0.667
	He	0.497	0.694	0.375	0.781	0.601	0.715	0.351	0.392	0.500
	F_{IS}	0.832*	−0.440	0.111	0.040	0.029	0.068	−0.188	−0.274	−0.333

Table 3. Private allelic richness for nine neutral microsatellite loci per population. Site names highlighted in black denote farmed corals. All other sites were natural populations.

Ayamaru	*Kuninao*	*Sesoko*	*Maeganeku*	*Onna*	*Maeda*	*Isa*	*Chibishi*	*Kume*
0.10	0.19	0.16	0.61	0.09	0.10	0.10	0.12	0.19
Ikema	*Shigira*	*Hirakubo*	*Nakano*	*Haemida*	*Taketomi*	*Kuroshima*	*Amitori*	
0.17	0.06	0.05	0.11	0.03	0.05	0.10	0.24	

nurseries, several ramets were abundant as a result of artificial fragmentation. However, it may not be necessary to cull these clonal colonies, because various wild populations in other areas have similar levels of clonal richness (Underwood 2009). Moreover, *Acropora* species are colonial modular organisms. Additionally, in coral nurseries, coral colonies can be arranged so as to increase fertilization rates, by placing genetically different colonies adjacent to one another. This is now being done, based on our earlier results allowing detection of clonal colonies (Omori et al. 2016). It should also be noted that Maeganeku Farm was estimated to have the highest private allelic richness of all sampling sites. Genetic isolation and networks between populations are essential for evolution. To avoid reduced genetic diversity in the future, genotypes of all farmed corals should be determined so as to guide fragmentation, as recommended by Baums (2008) and Johnson et al. (2011). Moreover, tracking of genotypes can be useful for selective breeding of strains that are resilient to environmental threats (Lundgren et al. 2013), including high water temperature, diseases, UV exposure, and sedimentation.

High values of inbreeding coefficients at several loci were observed not only in nurseries but also in most natural populations of *A. tenuis* in the Nansei Islands (Zayasu et al. 2016). However, heterozygote deficits may not be due to asexual reproduction because we did not find any clonal colonies in natural populations. Deviations from H–W expectations and high inbreeding coefficients F_{IS} values are surprisingly common among marine invertebrate species with planktonic sperm (Addison & Hart 2005). Acroporid corals release egg-sperm bundles, and high F_{IS} values have been reported previously for populations of *Acropora cytherea* and *A. hyacinthus* (Ayre & Hughes 2000; Marquez et al. 2002), *A. microphthalma* and *A. valida* (Richards & van Oppen 2012), and *A. digitifera* (Nakajima et al. 2010).

Coral nursery populations in Okinawa do not differ in genetic composition from wild populations in the Nansei Islands, although this is not surprising because farmed corals in the nurseries were obtained locally. Two wild populations (green and red) are observed in the Nansei Islands, with green predominating in the southwestern islands and red predominating in the northeastern part of the archipelago. The Chibishi Islands are the potential point of contact for these two populations (Zayasu et al. 2016). Corals in both nurseries were red-dominant, as at other sites in the northern Nansei Islands.

Due to the development of molecular resources during the past two decades, it has become possible to analyze reef connectivity (reviewed by Hellberg 2007). Several studies have revealed that panmixia occurs at relatively small spatial scales (reviewed by van Oppen & Gates 2006). Recent studies of *A. digitifera* (Shinzato et al. 2015) and *A. tenuis* (Zayasu et al. 2016) in Okinawa and northern Western Australia (Underwood 2009), *A. hyacinthus* around Palau (Cros et al. 2016), and *Acropora cervicornis* in the Florida Reef Tract (Drury et al. 2016) have revealed that population dynamics in archipelagos occur at more regional scales than was previously thought. Because there is potentially adaptive variation in response to

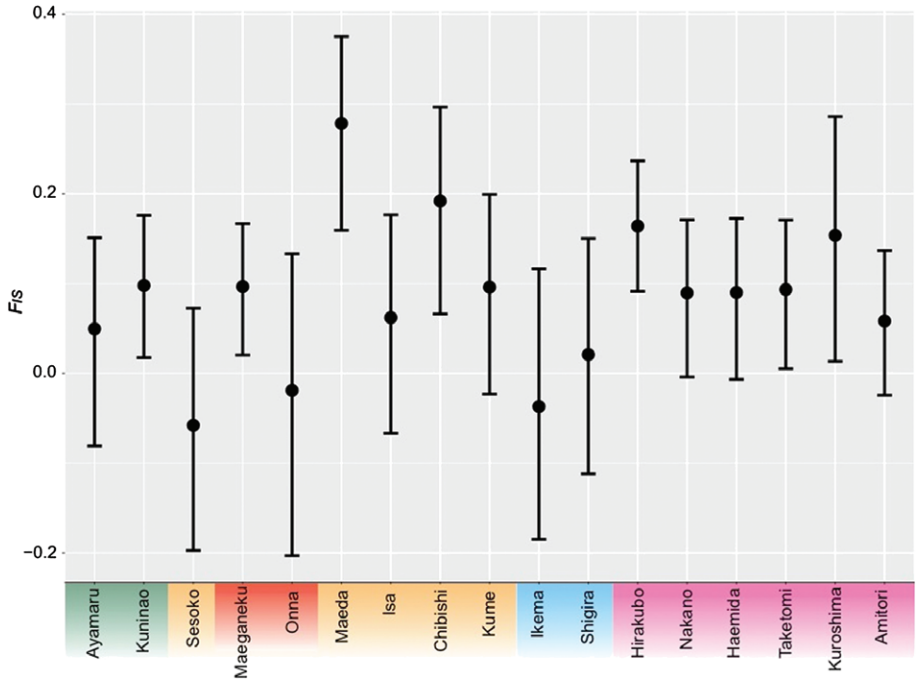

Figure 4. Inbreeding coefficient (F_{IS}) per population calculated from nine neutral microsatellite loci. Error bars are 95% CI. Colors of location names indicate island groups, as in Figure 1B.

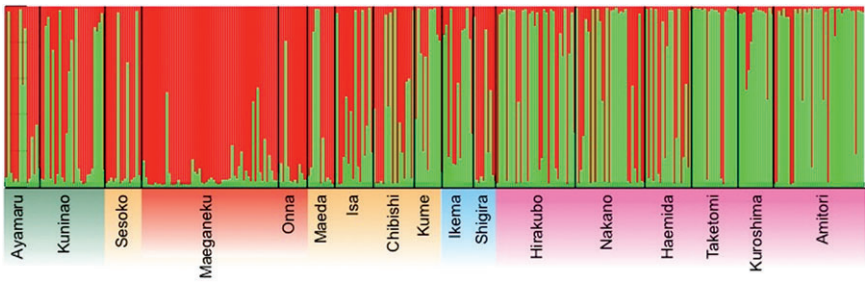

Figure 5. Estimated population structure and ancestral membership coefficients for all 369 genets (individuals), using $K = 2$. Thin vertical bars represent individuals, partitioned into K-colored segments (red or green) that represent the estimated membership fractions. Solid black lines separate sampling locations. Colors of location names indicate island groups, as in Figure 1B.

slight environmental differences between reefs, it is important that reef restoration decisions be data-driven, based on an understanding of genetic variation within and among populations. Moreover, the percentage of colonies arising by fragmentation varies by species (Baums 2008). In conclusion, the selection of suitable restoration methods according to species and objectives (short term or long term) will increase the success of effective restoration activities, because both reproductive strategy and spatial structure differ, depending on the species and local environmental characteristics. Further studies should investigate the genetic diversity of other coral species in the nurseries.

Coral nurseries in Okinawa not only contribute to asexual propagation but also production of gametes, due to massive synchronized spawning (Zayasu & Shinzato 2016). Moreover, transplanted corals may further enhance coral recruitment and natural recovery where recovery is slow or limited (Clark & Edwards 1995; Montoya Maya et al. 2016).

Obviously, reef impact mitigation should not replace reef protection as the first management option (Abelson 2006). With proper techniques, however, farmed corals may exhibit faster growth, and higher larval survivorship and attachment rates than wild corals (Higa & Omori 2014; Dela Cruz et al. 2015; Higa et al. 2017). This is probably due to less predation and sedimentation. Coral nurseries in which corals have higher survivorship than natural populations can function as gene banks. We believe that accurate genetic evaluation will become one of the most valuable tools for successful coral reef restoration.

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Detailed data for clonal colonies of *Acropora tenuis* in coral nurseries at Okinawa, Japan.

Table S2. Pairwise F_{ST} values among sites.

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