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REPORT

Regional genetic differentiation among northern high-latitude island populations of a broadcast-spawning coral

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Abstract Knowledge of genetic connectivity is useful for understanding of the recovery potential of coral populations after various disturbances, such as coral mass bleaching. Population genetic studies in corals are mostly restricted to Australian and Caribbean species; studies in the northern Pacific are relatively limited. Using microsatellite markers, the population genetics of *Acropora* sp. 1 was examined between two regions in Japan, the Okinawa-Aka and Bonin Islands, which are separated by approximately 1,500 km of open water in a high-latitude area. Statistically significant but small genetic differentiation in *Acropora* sp. 1 was detected between and within these regions. Genetic diversity was not obviously reduced in populations of the Bonin Islands, which are relatively isolated. Thus, some level of connectivity appears to be maintained between the two regions, likely because of the high dispersal ability of this broadcast spawner.

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Introduction

Oceanic environmental changes, such as increasing sea surface temperatures, ocean acidification, and various local disturbances (e.g., sediment pollution or nutrient influx), are expected to threaten the survival and growth of reef-building corals (e.g., Hoegh-Guldberg 1999; Bellwood et al. 2004; Hoegh-Guldberg et al. 2007; Cantin et al. 2010). To estimate how coral populations will be affected by future environmental changes, it is essential to understand their metapopulation structure over large geographic ranges. For marine organisms, recruitment is essential for adding new individuals to populations and for maintaining successive life cycle stages within populations (Caley et al. 1996). Thus, knowledge of dispersal distances and pathways is useful for understanding of recovery potential in coral populations (van Oppen et al. 2008). Many marine species have limited to no adult movement. For these species, the larval stages provide the main opportunities for species dispersal (Hellberg 2009). Among broadcast-spawning coral species, the pelagic larvae are responsible for most of the connectivity among populations. New recruitment from locally and externally sourced larval stages, as well as regrowth of surviving coral colonies and colony fragments, strongly contributes to the recovery of coral populations (van Oppen et al. 2008).

Although pelagic larvae of marine organisms sometimes disperse over very long distances, simple direct tracking of larval trajectories from spawning to settlement is not easily accomplished (Palumbi 2003). Furthermore, such tracking is inadequate for quantifying population connectivity

because connectivity is reduced by natural selection in environments with exogenous colonization (Pineda et al. 2007; Marshall et al. 2010). Population genetic studies in various marine invertebrates provide an alternative method for estimating larval dispersal (Hellberg 2009; Weersing and Toonen 2009). Several population genetic analyses of corals have been undertaken (reviewed in van Oppen and Gates 2006). Those using highly variable genetic markers such as microsatellites have recently become the norm in examination of reef-building coral species (e.g., Baums et al. 2005a, 2006; Magalon et al. 2005; van Oppen et al. 2008; Nakajima et al. 2009a, 2010). Most population genetic studies in corals have been performed in the Southern Hemisphere such as Australia and a part of the Northern Hemisphere such as the Caribbean Sea. Work in the northern Pacific has been relatively limited, and microsatellite marker procedures have rarely been used in this sector of the world's oceans.

Reduced genetic connectivities between populations and genetic diversity within populations are expected in high-latitude and marginal coral populations because the conditions they encounter are more extreme than those in their natal habitats (e.g., Ayre and Hughes 2004; Ridgway et al. 2008; Noreen et al. 2009). However, a few studies focusing on high-latitude populations, including marginal populations, have tended to demonstrate that connectivity is maintained (e.g., Nakajima et al. 2010). Thus, a clear consensus does not exist as to whether genetic connectivity is reduced or maintained in high-latitude and marginal coral populations.

Few studies of coral population genetics have been conducted in the southwestern areas of Japan, where many coral species reach their northern distribution limits under the prevailing subtropical and temperate zones (Nishihira and Veron 1995). In the Ryukyu Archipelago, which is located in southeastern Japan, population genetics have examined five coral species (*Acropora digitifera*, *Acropora tenuis*, *Goniastrea aspera*, *Pocillopora damicornis*, and *Stylophora pistillata*) using allozyme electrophoresis (Adjeroud and Tsuchiya 1999; Nishikawa et al. 2003; Nishikawa and Sakai 2003, 2005). These studies have demonstrated that the extent of genetic differentiation is different among species; broadcast-spawning corals have especially high genetic connectivity among regions. However, other than the study by Nakajima et al. (2010), large-scale studies using microsatellite markers have never been undertaken in this area.

Okinawa Island and the Bonin Islands are located at almost the same latitude (Fig. 1), but are separated by about 1,500 km of open water. *Acropora* is a dominant coral genus in the Indo-Pacific region; it is highly susceptible to bleaching (van Oppen et al. 2001; McClanahan et al. 2007). In the Nansei Islands, including Okinawa Island, the broadcast spawner *A. digitifera* is a dominant coral even though the area represents a high-latitude and peripheral habitat for this species. We demonstrated previously that a high genetic connectivity exists among *A. digitifera* populations in the Nansei Islands (Nakajima et al. 2010). This connectivity is thought to be maintained by stepping-stone gene flow through reefs located near separated islands, and the Kuroshio Current, which flows from south to north through the Nansei Islands. In contrast, the Bonin Islands are far from the Kuroshio Current, and few reefs exist in the stretch of open sea to Okinawa Island. Thus, we hypothesized that genetic differentiation would be detectable between some Okinawa Island and Bonin Islands sites. Furthermore, we hypothesized that genetic diversity would be reduced in isolated populations of the Bonin Islands. We selected *Acropora* sp. 1 aff. *digitifera* (hereafter called "*Acropora* sp. 1") (Hayashibara and Shimoike 2002), a cryptic species of *A. digitifera*, as a target species because it is frequently found at both Okinawa Island and the Bonin Islands; *A. digitifera* has rarely been found around the Bonin Islands. A significant genetic break exists between populations of the two species occurring in sympatry (Nakajima et al. 2012). Currently, the habitat range of *Acropora* sp. 1 is not well defined. Furthermore, we aimed to compare patterns of genetic differentiation in *Acropora* sp. 1 with our previous result in *A. digitifera*. *A. digitifera* spawns in the period May–June (main simultaneous spawning season in genus *Acropora*), but *Acropora* sp. 1 spawns mostly in August at Aka-jima Island (Okinawa region) (Hayashibara and Shimoike 2002). In spite of this difference, most biological features of *Acropora* sp. 1 are very similar to those of *A. digitifera*, including the shape (corymbose type), reproductive mode (broadcast-spawning), and habitat conditions (wave-exposed shallows); therefore, an interspecific comparison is appropriate.

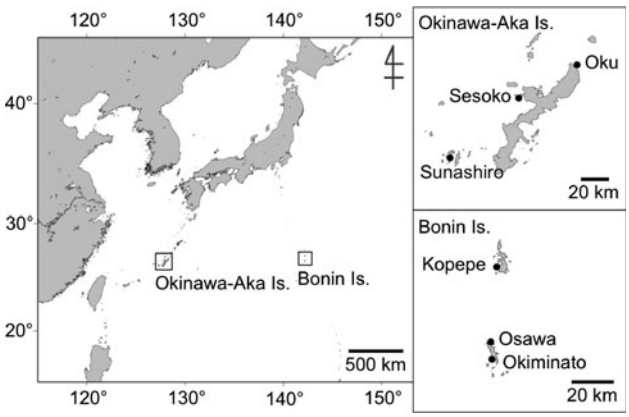

Fig. 1 Map of the Okinawa-Aka and Bonin Islands showing the six sampling sites

Materials and methods

Sampling and genomic DNA extraction

Sampling sites were established at six localities at Okinawa and Aka-jima Island (hereafter called "Okinawa-Aka Islands") and the Bonin Islands, which are in the southern sector of Japan (Fig. 1). We selected two sites from Okinawa Island and one from Aka-jima Island. In addition, we selected two sites from Haha-jima Island and one from Chichi-jima Island in the Bonin Islands. Across all sample localities, we haphazardly collected fragments from a total of 211 *Acropora* sp. 1 colonies that were >3 m apart; one fragment was taken from each colony. Fragments were preserved in 100 % ethanol in 1.5-ml Eppendorf tubes and transported to the laboratory. Genomic DNA was extracted from coral surface tissues using the AquaPure Genomic DNA kit (Bio-Rad, Hercules, CA, USA).

Genotyping

We adapted primers for microsatellite markers that were developed for *A. digitifera* by Nakajima et al. (2009b). We also adapted primers that were developed for *Acropora palmata* by Baums et al. (2005b) and *Acropora millepora* by van Oppen et al. (2007); these primers were also suitable for *Acropora* sp. 1 (Nakajima et al. 2009b). We amplified microsatellite regions with multiplex PCR method (adding two primer sets to one PCR) using Ex Taq DNA polymerase (Takara, Tokyo, Japan) with 10× Ex Taq buffer, 4 pM dNTPs (1 pM each), 100 nM primers (for two loci), 0.125 U Ex Taq DNA polymerase, about 5 ng μl^{-1} (multiC: 1 ng μl^{-1}) template DNA, and MilliQ water (Millipore, Billerica, MA, USA) for a total reaction volume of 5 µl. Amplifications were carried out in a PC-818 touchdown thermocycler (Astec, Chattanooga, TN, USA) operated under the following conditions: 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, 50 °C (gradient: − 0.1 °C $cycle^{-1}$) for 30 s, 72 °C for 1 min, and a final 72 °C extension for 30 min. Allelic variations were analyzed using a DNA capillary sequencer CEQ-8800 (Beckman Coulter, Fullerton, CA, USA). When alleles were unclear or not detected, normal PCR (i.e., not multiplex) was conducted using 100 nM primers for one locus (forward and reverse primers were each 50 nM). We found no shifts in allele size between multiplex and normal PCR.

Statistical analyses

The numbers of alleles, allele frequencies, observed heterozygosity (H_o) and expected heterozygosity (H_e), number of private alleles, and inbreeding coefficient (F_{IS}) values were calculated using GenAlEx software (ver. 6.4; Peakall and Smouse 2006). F_{IS} values were used to judge deviations from Hardy–Weinberg equilibrium (HWE) because gaps between H_o and H_e under HWE are in proportion to the values of the F_{IS}. That is, positive and negative F_{IS} values suggest deficits and excesses of heterozygosity, respectively. An exact test for departure from HWE was also performed. For F_{IS} values, statistical significance levels were $P < 0.05$ after adjusting for multiple comparisons using a false discovery rate (FDR) correction following Benjamini and Hochberg (1995). Allelic richness (average number of alleles) was calculated as an index of genetic diversity, standardized to 11 colonies analyzed (the smallest sample size), using the FSTAT software (ver. 2.9.3.2; Goudet 1995).

The extent of asexual reproduction was estimated from the genotypic richness of each population using GenAlEx software. If several unique multilocus genotypes were detected, *Ng* was used as an estimate of the minimum number of clones present in a population. Where *N* indicates the number of genotyped individual colonies, *Ng*/*N* provides an index of the effects of asexual reproduction and suggests genotypic richness (Coffroth and Lasker 1998). Values of *Ng*/*N* were high regardless of regions or sites. When $Ng/N = 1$, all of the collected colonies in a population are unique (no clones); *Ng*/*N* approaches zero when a population has only a single genotype (all clones).

To measure the proportion of genetic variation between sites, we used *F*-statistics via analysis of molecular variance (AMOVA; Excoffier et al. 1992). This analysis was carried out using GenAlEx software to test the significance of all estimates based on 999 random permutations. A low pairwise F_{ST} indicates high gene flow and vice versa. Statistical significance levels for all pairwise tests were $P < 0.05$ after adjusting for multiple comparisons using a FDR correction. Pairwise geographic distances between sites were also calculated by GenAlEx software to estimate the scale of populations analyzed. A plot of isolation-by-distance (IBD) was used to explore the relationship between the calculated geographic distance and $(1 - F_{ST})/F_{ST}$ as a genetic differentiation index. The significance of IBD was tested by a Mantel test (Mantel 1967) using GenAlEx software.

Population structure was inferred from microsatellite data using STRUCTURE software (ver. 2.3.1; Pritchard et al. 2000). This software applies a Bayesian clustering approach to identify populations possessing a characteristic set of allele polymorphisms based on genotyping data for microsatellite alleles. A burn-in period of 100,000 followed by 1,000,000 Markov chain Monte Carlo replications was used for population clustering, with prior information under the admixture model and assuming correlated allele frequencies (Falush et al. 2003). Simulations included 20 iterations for each *K* value ($K = 1$–9), and mean Ln

P(D) values were calculated. From these values, ΔK values for $K = 2$–8 were calculated using the method of Evanno et al. (2005). The ΔK value is an index for deciding the probable number of genetically clustering populations (K), and we determined that K at the highest ΔK was the most probable number of clustering populations. Plot data were generated by CLUMPP software (ver. 1.1.2b; Jakobsson and Rosenberg 2007). Migration patterns and rates among sites were estimated using BAYESASS software (ver. 1.2; Wilson and Rannala 2003) with default settings [chain length (3×10^6 iterations) and a burn-in period (1×10^6 iterations)].

Results

We obtained 192 complete multilocus genotypes from a total of 211 coral fragments of *Acropora* sp. 1. The STRUCTURE analysis estimating the most probable number of genetically clustering populations (K) indicated the existence of a clear genetic population structure between the Okinawa-Aka and Bonin Islands. When K was 2, the value of ΔK was largest [$\Delta K = 3.42$, Ln P(D) = −3613.87], and the plotted data demonstrated population differentiation between the Okinawa-Aka and Bonin Islands. Only the Sesoko population had an intermediate level of differentiation between the two regions (Fig. 2).

Values of mean allelic richness for all loci ranged from 5.50 to 6.28 (Table 1). Mean H_o and H_e values for all loci across sites ranged from 0.470 to 0.628 and 0.527 to 0.603, respectively, and the total mean values were 0.536 ± 0.053 (±SD) and 0.578 ± 0.024 (±SD), respectively (Table 1). Allelic richness and heterozygosity were similar across populations within the Bonin Islands. A total of 16 private alleles were detected across all loci and regions. Private alleles were observed in all sites except Kopepe, where the number of samples collected was small ($N = 11$). Eleven private alleles were found in the Okinawa-Aka Islands ($N = 114$), and five were observed in the Bonin Islands ($N = 78$). Departures in population heterozygosity from HWE were indicated by mean F_{IS} values, which ranged from −0.060 to 0.216 at all sites (Table 1); however, F_{IS} values were not available for MS8 and A.mill2–8 at some sites because of fixation of an allele (Table 2). The ratio of the number of observed multilocus genotypes (Ng) to the number of colonies analyzed (N) at sites ranged from 0.93 to 1.00, with an overall value of 0.97 (Table 1). Therefore, levels of clonality were low in populations of *Acropora* sp. 1 we sampled.

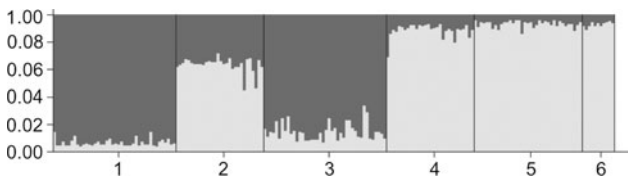

Fig. 2 *Bar plot* of a Bayesian-based clustering analysis implemented by STRUCTURE software. *1* Oku, *2* Sesoko, *3* Sunashiro, *4* Osawa, *5* Okiminato, *6* Kopepe. This panel shows an analysis of two genetically clustered populations [$K = 2$, mean Ln P(D) = −3613.87]; output from CLUMPP software

The AMOVA calculated variance values of 0.026 (1 %) among regions, 0.019 (1 %) among populations, and 1.777 (98 %) within populations (total value: 1.822); a significant difference was detected among populations ($P < 0.001$) (Table 3). Pairwise F_{ST} values across regions ranged from 0.015 to 0.043. Although these values indicate low levels of genetic differentiation, all pairwise F_{ST} values between regions were statistically significant (Table 4). Within the Bonin Islands, no significant F_{ST} values were detected between sites ($F_{ST} = 0.001$–0.015; distances ranged from 5.8 to 46.4 km). However, statistically significant but small F_{ST} values were observed (Oku-Sesoko: $F_{ST} = 0.024$, 48.4 km; Sesoko-Sunashiro: $F_{ST} = 0.019$, 76.7 km) between sites within the Okinawa-Aka Islands, despite the small distances (48.4–124.3 km) separating these sites relative to the geographic scale (about 1,500 km) of the study. We detected a significant IBD in *Acropora* sp. 1 at the geographic scale of the study, although the calculated P value was close to the significance threshold and the R^2 value was not high ($P = 0.030$ and $R^2 = 0.37973$; Fig. 3). Our BAYESASS analysis detected a high degree of migration within regions in some cases (Table 5). In the Okinawa-Aka Islands, we detected migrant patterns from Sunashiro to two other regions (into Oku, 27.64 %; into Sesoko, 9.68). In the Bonin Islands, similar patterns were found at Okiminato (into Osawa, 26.91 %; into Kopepe, 22.59 %). We also detected a migration pattern between regions (into Sesoko, Okinawa from Okiminato, Bonin: 19.57 %).

Discussion

The high values of Ng/N (0.93–1.00 for each site, mean: 0.97) suggest that the low level of genetic differentiation among populations is maintained by the dispersal of sexually produced planula larvae and not by asexual reproduction through fragmentation. Furthermore, high allelic richness and heterozygosity have been maintained in both regions. The number of private alleles ranged from two to six for each of the six loci at five of the study sites; the exception was the Kopepe site, in which the sample size was small ($N = 11$). The existence of private alleles at almost all of the sites suggests that a large effective population facilitates the maintenance of low-frequency alleles in the population.

Bayesian clustering analysis showed two differentiated clusters between the two regions: one cluster included Oku and Sunashiro in the Okinawa-Aka Islands, and the second

Table 1 Number of colonies analyzed *N*, Number of multilocus genotypes (*Ng*), *Ng*/*N* ratio for estimating the contribution of sexual/asexual reproduction, mean observed (H_o) and expected (H_e) heterozygosity, mean inbreeding coefficients (F_{IS}), allelic richness (average number of alleles, standardized to 11 colonies analyzed), and the number of private alleles for all six loci at six sampling sites

Region	Site	Location	*N*	*Ng*	*Ng*/*N*	Mean H_o	Mean H_e	Mean F_{IS}	Allelic richness	Private alleles
Okinawa-Aka	Oku	26°50′N 128°17′E	42	40	0.95	0.532	0.582	0.057	6.13	3
	Sesoko	26°38′N 127°51′E	30	28	0.93	0.628	0.603	−0.060	6.13	2
	Sunashiro	26°11′N 127°16′E	42	42	1.00	0.512	0.575	0.091	6.26	6
Bonin	Osawa	26°41′N 142°08′E	30	29	0.97	0.578	0.593	0.001	6.28	3
	Okiminato	26°38′N 142°09′E	37	37	1.00	0.495	0.587	0.216	5.92	2
	Kopepe	27°03′N 142°11′E	11	11	1.00	0.470	0.527	0.095	5.50	0
	Total or mean (±SD)		192	187	0.97	0.536 ± 0.053	0.578 ± 0.024	0.067 ± 0.086	6.04 ± 0.267	2.67

Allelic richness was calculated using FSTAT software; other calculations were performed using GenAlEx software

included Osawa, Okiminato, and Kopepe in the Bonin Islands. However, the differentiation level of the Sesoko population indicated some degree of admixture between both clusters on the output panel. This admixture may have arisen because differentiation was not large and connectivity was maintained between two regions by geographic and environmental factors. The genetic differentiation between two regions that we detected was a low level; pairwise F_{ST} values for all site combinations were less than 0.044. However, all F_{ST} values between regions were statistically significant. In our analysis of migration patterns, we detected migration between regions in one case (from Okiminato, Bonin, into Sesoko, Okinawa); otherwise, most migration events occurred within regions. The IBD graph also suggests genetic differentiation between the two regions (statistically significant *P* value); however, the R^2 value was not high. We believe that several factors, including geographic distances, are reflected in the result of IBD. Biological features, such as larval mortality and the existence of populations recovering after disturbance, may make interpretation of the IBD result more complex.

The occurrence of small but statistically significant genetic differentiation between the Okinawa-Aka and Bonin Islands might have arisen because few shallow reefs exist between the two regions, which are separated by about 1,500 km. In contrast to the results for *Acropora* sp. 1, our previous study demonstrated that in *A. digitifera*, little significant differentiation exists among populations separated by approximately 1,000 km within the Nansei Islands (Nakajima et al. 2010). Small-scale genetic heterogeneity and broad-scale homogeneity have been detected in some species, including brooders and spawners in the Nansei Islands (Nishikawa 2008). The extent of genetic differentiation appears to depend on the reproductive modes of coral species in question (Nishikawa et al. 2003), but F_{ST} values are also variable among species within same genus even though they share reproductive modes (Nishikawa and Sakai 2005). Thus, F_{ST} differences may be shifted by biological parameters of life stages other than those of the planktonic larva, including fecundity, fertilization rate, mortality, and resilience. However, details of biological trait differences between *A. digitifera* and *Acropora* sp. 1 are not yet available.

The differences between findings of the present work and those of our previous study on *A. digitifera* may be largely attributable to differences in geographic factors (isolated islands in this study vs. continuous islands in the previous study) and geographic scales (about 1,500 km vs. about 1,000 km). Mukai et al. (2009) reported that reef goby (*Bathygobius cocosensis*) population in the Bonin Islands is genetically divergent from three populations at Wakayama (Japan), Okinawa (Japan), and Guam (US pacific territory), but the three latter populations are genetically uniform. The genetic panmixia of *B. cocosensis* in the Wakayama, Okinawa, and Guam populations implies that the North Equatorial and Kuroshio Currents are powerful transporters of fish larvae in the northwestern Pacific Ocean. In contrast to these populations, genetic differentiation between the Bonin Islands and the Wakayama, Okinawa, and Guam populations suggests that ocean currents transport few larvae of this species to the Bonin Islands (Mukai et al. 2009). Our results reported here are

Table 2 Number of colonies analyzed (*N*), number of alleles (*Na*), observed (H_o) and expected (H_e) heterozygosity, inbreeding coefficients (F_{IS}), and the number of private alleles for each locus at six sampling sites

Pop	MS166	MS181	MS182	MS8	A.mill2–8	A.mill2–22
Oku ($N = 42$)						
Na	13	16	14	3	2	6
H_o	0.762	0.690	0.810	0.095	0.190	0.643
H_e	0.853	0.883	0.878	0.092	0.210	0.575
F_{IS}	0.107	**0.218**	0.078	−0.037	0.092	−0.119
Private alleles		2		1		
Sesoko ($N = 30$)						
Na	11	14	15	1	4	5
H_o	0.867	0.733	0.900	0.000	0.400	0.867
H_e	0.798	0.893	0.892	0.000	0.372	0.662
F_{IS}	−0.086	0.179	−0.009	N/A	−0.076	−0.310
Private alleles		1			1	
Sunashiro ($N = 42$)						
Na	12	19	14	2	4	7
H_o	0.810	0.619	0.833	0.024	0.167	0.619
H_e	0.866	0.905	0.878	0.024	0.215	0.564
F	**0.065**	**0.316**	0.050	−0.012	0.223	**−0.097**
PVA		3	1		1	1
Osawa ($N = 30$)						
Na	12	12	17	1	2	5
H_o	0.967	0.700	0.900	0.000	0.233	0.667
H_e	0.891	0.878	0.912	0.000	0.206	0.671
F_{IS}	−0.085	0.203	0.013	N/A	−0.132	0.006
Private alleles			3			
Okiminato ($N = 37$)						
Na	10	13	15	1	2	7
H_o	0.784	0.514	0.865	0.000	0.081	0.730
H_e	0.854	0.865	0.907	0.000	0.214	0.680
F_{IS}	0.083	**0.407**	0.046	N/A	**0.621**	−0.074
PVA		1				1
Kopepe ($N = 11$)						
Na	10	8	10	1	1	3
H_o	0.909	0.545	0.636	0.000	0.000	0.727
H_e	0.884	0.769	0.880	0.000	0.000	0.628
F_{IS}	−0.028	0.290	0.277	N/A	N/A	−0.158
Private alleles						
All						
Na	15	24	21	3	5	9
F_{IS}	0.010	0.268	0.075	−0.032	0.119	−0.125

Bold F_{IS} values indicate significant differentiation ($P < 0.05$) after adjusting for multiple comparisons using a false discovery rate (FDR) correction

concordant with those of Mukai et al. (2009) in that we observed genetic differentiation between regions that are not connected by strong ocean currents; the Bonin Islands are isolated from both typical and large paths of the Kuroshio Current (Miyazawa et al. 2004). However, the low pairwise F_{ST} values and higher genetic diversity in the isolated population suggests that larval dispersal may occur between the Okinawa-Aka and Bonin Islands. Noreen et al. (2009) indicated that infrequent successful long-distance dispersal of the brooding coral *Seriatopora hystrix* from the Great Barrier Reef supplements high-latitude reefs at Lord Howe Island. Considering the higher dispersal ability of

Table 3 Hierarchical analysis of molecular variance (AMOVA) analysis using GenAlEx software

Source	*d.f.*	Sum of squares	Est. Var.	% of the total variances	*P*
Among regions	1	7.901	0.026	1	<0.001
Among populations	4	11.794	0.019	1	<0.001
Among individuals within populations	378	671.795	1.777	98	<0.001
Total	383	691.490	1.822		

Table 4 Pairwise F_{ST} values for all six populations as indices of genetic differentiation (below diagonal) and pairwise geographic distance (km) based on geographic coordinates of sampling sites (above diagonal)

	Oku	Sesoko	Sunashiro	Osawa	Okiminato	Kopepe
Oku		48.4	124.3	1374.5	1376.6	1377.4
Sesoko	**0.024**		76.7	1418.7	1420.6	1422.1
Sunashiro	0.001	**0.019**		1480.5	1482.3	1485.1
Osawa	**0.033**	**0.015**	**0.027**		5.8	41.1
Okiminato	**0.022**	**0.015**	**0.017**	0.001		46.4
Kopepe	**0.041**	**0.036**	**0.043**	0.015	0.015	

Bold values indicate significant differentiation ($P < 0.05$) after adjusting for multiple comparisons using a false discovery rate (FDR) correction. Both pairwise F_{ST} and geographic distances were calculated using GenAlEx software

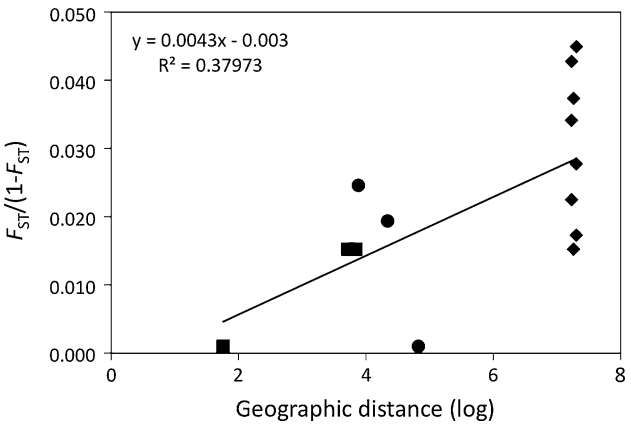

Fig. 3 Isolation-by-distance (IBD) estimated from pairwise $F_{ST}/(1 - F_{ST})$ values for six populations plotted against geographic distance (*circle*: within Okinawa-Aka; *square*; within Bonin; *diamond*: between Okinawa-Aka and Bonin)

Acropora sp. 1 in comparison with *S. hystrix*, even infrequent larval recruitment by *Acropora* sp. 1 to the Bonin Islands from source populations in the Okinawa-Aka Islands is likely sufficient to maintain connectivity between these two regions.

Significant differentiation in pairwise F_{ST} values was found within the Okinawa-Aka Islands, but no significant differentiation within the Bonin Islands was detected. The difference in genetic differentiation between the two regions may be attributable to hydrography or immigration events in each of them. A similar lack of pattern concordance between geographic distance and genetic differentiation within small-scale systems occurs in other broadcast-spawning coral species as well (e.g., Magalon et al. 2005; Nakajima et al. 2009a, 2010); some large-scale analyses indicate that currents are the main factor influencing genetic differentiation (e.g., Baums et al. 2006).

Table 5 Migration values estimated by the Bayesian assignment test using BAYESASS software

Into	From Okinawa-Aka			From Bonin		
	Oku	Sesoko	Sunashiro	Osawa	Okiminato	Kopepe
Oku	**68.57 (1.86)**	0.52 (0.68)	**27.64 (3.55)**	0.52 (7.36)	2.33 (2.80)	0.42 (0.59)
Sesoko	0.76 (1.05)	**68.58 (2.17)**	**9.68 (7.58)**	0.78 (1.08)	**19.57 (8.36)**	0.63 (0.87)
Sunashiro	0.90 (1.33)	1.02 (1.32)	**91.81 (5.24)**	1.02 (1.24)	4.75 (4.59)	0.50 (0.77)
Osawa	0.84 (1.20)	0.67 (0.96)	2.91 (2.71)	**68.15 (1.44)**	**26.91 (3.49)**	0.52 (0.74)
Okiminato	0.30 (0.55)	0.29 (0.56)	0.63 (1.37)	0.27 (0.55)	**98.29 (2.03)**	0.22 (0.40)
Kopepe	1.97 (2.57)	1.50 (2.11)	2.81 (3.76)	1.86 (2.52)	**22.59 (5.85)**	**69.26 (2.54)**

The values show the estimated percentages of individuals that migrated into each site in the Okinawa-Aka and Bonin Islands. Values in brackets indicate standard deviations. Bold numbers indicate the representative migration values (>5 %)

The relationship between present-day current patterns and inferred patterns of gene flow is usually unknown (Hellberg 2007), but studies in the northwestern Pacific have demonstrated such a relationship (e.g., Ravago-Gotanco et al. 2007; Yasuda et al. 2009). Factors other than ocean currents likely influence patterns of connectivity. For example, larval survival probability during dispersal is dependent on food availability (Pineda et al. 2007), predation, and mass bleaching events (Magalon et al. 2005). Coral populations in both the Okinawa-Aka and Bonin Islands were reduced by a mass bleaching event in 1998; parts of the populations have not yet recovered (Ministry of the Environment of Japan 2004; Ministry of the Environment of Japan and Japanese Coral Reef Society 2004). Differences in recovery speed between coral populations may reflect differences in genetic differentiation.

Previous studies have demonstrated depressed genetic connectivity and diversity in high-latitude and marginal populations (Ayre and Hughes 2004; Ridgway et al. 2008; Noreen et al. 2009), and such populations are believed to rarely recover after environmental disturbance. In contrast, we found that the genetic connectivity of *Acropora* sp. 1 was maintained between the two regions we studied and that genetic diversity was not depressed at these high-latitudes, as we showed in our previous study (Nakajima et al. 2010). In some coral species, slight recruitment on rare occasions has the potential to maintain genetic diversity among marginal populations (Noreen et al. 2009; Nunes et al. 2011). Furthermore, a previous study by Nunes et al. (2009) indicated that genetic diversity is maintained in northern marginal population of the Caribbean/North Atlantic, but reduced in two other marginal populations of the coral *Montastraea cavernosa* in the western South Atlantic (Brazil) and eastern Tropical Atlantic (West Africa). Nunes et al. (2011) indicated that the genetic diversity in broadcast-spawning species is higher than in brooding species, although broadcast-spawning species also have decreasing genetic diversity with increasing latitude at high-latitudes (Ridgway et al. 2008). Therefore, contradictions among studies appear to be attributable to the differences in geographic conditions and reproductive modes. Although populations in the Okinawa-Aka Islands appear to be influenced by the Kuroshio Current, as mentioned above, no strong current occurs around the Bonin Islands. If the pelagic larval phase of *Acropora* sp. 1 is as long as that of *A. digitifera*, which remains in suspension over 54 days under experimental conditions (Nishikawa and Sakai 2005), long-distance dispersal of *Acropora* sp. 1 may contribute to the low level of genetic differentiation and high level of genetic diversity in the Bonin Islands. Such long-distance, but occasional, dispersal may be driven by weak currents over one or a few generation(s), as we demonstrated in our data on migration patterns.

Mass bleaching events, outbreaks of coral-eating starfish, and recent anthropogenic disturbances are also potential factors affecting the recruitment and maintenance of coral populations. In the future, analyses of corals using non-neutral markers that focus on phenotypic differences and zooxanthella genotyping may provide additional information that improves understanding of comprehensive reef connectivity. Similar genetic analyses on other coral genera (e.g., *Pocillopora*, *Porites*) will also provide useful information in establishing conservation strategies for coral reef ecosystems.

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