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Genetic Variation and Gene Flow of Broadcast Spawning and Planula Brooding Coral, *Goniastrea aspera* (Scleractinia) in the Ryukyu Archipelago, Southern Japan

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ABSTRACT—The scleractinian coral *Goniastrea aspera* (Verrill) undergoes both broadcast spawning and planulae brooding in the Ryukyu Archipelago of southern Japan. Genetic variation and gene flow in *G. aspera* were studied using allozyme electrophoresis. We tested the hypothesis that gene flow is determined by the competency period of the planulae. We also assessed the relative contributions of sexual and asexual reproduction to recruitment. For the five staining systems surveyed, *G. aspera* encoded five polymorphic loci and one monomorphic locus. The genotype frequencies in each population significantly differed from the expected Hardy-Weinberg equilibrium (HWE), indicating that the local populations of *G. aspera* are not fully panmictic. The high ratio of the observed number of genotypes to the number of individuals (0.90±0.07, mean N_G:N±SD) and the observed to expected genotypic diversity (0.84±0.11, mean G_O:G_E±SD) suggested that each population is likely maintained by sexual reproduction. The genetic differentiation (F_{ST}) and value of average number of migrants per generation (N_em) among and within regions ranged from 0.025 to 0.104 and 2.2 to 9.6, respectively. Comparisons with other species demonstrated that larva survival rates also influence gene flow. In addition, gene flow on distant reefs by planulae originating from spawning might prevent divergence by planulae originating from brooding for short-distant dispersal among and within populations of *G. aspera* in the Ryukyu Archipelago.

Key words: scleractinian coral, reproductive mode, gene flow, allozyme electrophoresis

INTRODUCTION

The effects of differences in reproductive modes on genetic variability and gene flow have been studied in some marine invertebrates, *i.e.*, gamete spawners or planulae brooders (Benzie and Williams, 1997; Ayre and Dufty, 1994; Ayre *et al.*, 1997a, b; Yu *et al.*, 1999; Ayre and Hughes, 2000; Bastidas *et al.*, 2001, 2002; Nishikawa *et al.*, in press). Several population genetic analyses using allozyme electrophoresis have shown that brooding species have restricted gene flow (N_em: number of migrants per generation) compared with spawning species in ascidians, scleractinian corals, and alcyonacean corals (Hellberg, 1996; Ayre *et al.*, 1997a; Bastidas *et al.*, 2001, 2002; Nishikawa *et al.*, in press). These reports are in good agreement with the characteristics of the reproductive mode; larvae produced by brooding generally settle quickly after their release from the parental colony, while larvae produce by spawning delay settling for at least a few days (*e.g.*, Svane and Young, 1989; Harrison and Wallace, 1990). By contrast, an allozyme study of five brooding and four spawning scleractinian coral species on the Great Barrier Reef (GBR) did not fully support this simple prediction (Ayre and Hughes, 2000), as it demonstrated that larval dispersal was sufficient to maintain moderate to high levels of gene flow along the entire GBR in three of five brooding species.

The scleractinian coral *Goniastrea aspera* (Verrill) is widely distributed in Indo-Pacific reefs. Abe (1937) and Motoda (1939) reported that *G. aspera* in Palau released brooded planulae. By contrast, *G. aspera* on the GBR is a hermaphroditic spawner that spawns once per year (Babcock, 1984; Harrison *et al.*, 1984; Willis *et al.*, 1985; Babcock *et al.*, 1986). In the Ryukyus, Sakai (1997) reported that individual colonies of *G. aspera* brooded planulae after spawning gametes. *Goniastrea aspera* broods planula, which is probably only capable of short distance dispersal since laboratory experiments have shown that brooded planulae settle within one hour of release. Nishikawa *et al.* (in press) compared larval settlement rates and gene flow in scleractinian corals, using electrophoresis to examine a spawner (*Acropora tenuis*) and a brooder (*Stylophora pistil-*

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lata) in the Ryukyu Archipelago. They estimated that the settlement-competency period in *A. tenuis* and *S. pistillata* was 69 and 51 days, respectively. Moreover, they also estimated that N_em in *A. tenuis* and *S. pistillata* was 16.4 and 1.5, respectively. They concluded that the longer competency period of *A. tenuis* larvae versus those of *S. pistillata* was one of the major cause of the difference in N_em. The estimated competency period of planulae originating from spawning in *G. aspera* in the Ryukyus was 63–70 days, similar to that of *A. tenuis* (Nozawa, 2000). These results suggest that planulae originating from spawning in *G. aspera* have the potential to disperse long distances. Due to the unusual reproductive mode of *G. aspera* in the Ryukyus, we hypothesized that gene flow in *G. aspera* would exceed that in the brooding coral *S. pistillata* and not differ greatly from that in the spawning coral *A. tenuis*. Although brooded larvae may display restricted gene flow among and within populations due to their short-distance dispersal, the recruitment of planulae originating from spawning on distant reefs might prevent the fixation of genetic differences.

In Western Australia, the brooded planulae of the viviparous coral *Pocillopora damicornis* are thought to be derived from asexual reproduction (Stoddart, 1983, 1984a, b). He reported that larvae of *P. damicornis* are genotypically identical to their brood parents; local populations are dominated by a small number of clones (highly replicated multi-locus genotypes), which are generated asexually. By contrast, there was no evidence of asexual replication of genotypes in *P. damicornis* on the GBR (Benzie *et al*., 1995; Ayre *et al*., 1997b). The relative importance of sexual and asexual reproduction has profound effects on the genetic structure of populations (Jackson, 1986). The occurrence of asexual reproduction can lead to gene frequencies that are skewed from those expected under random mating. In addition, clonal life should also accelerate evolutionary change within, and divergence among, local populations (Wright, 1978). For *G. aspera* in the Ryukyus, Sakai (1997) suggested that brooded planulae are very likely the products of sexual reproduction, because eggs remained in the polyps after spawning and there was no sign of asexual reproduction. However, population genetic data on *G. aspera* in the Ryukyu has been limited.

It should be noted that indirect estimates of genetic differentiation (F_{ST}) may low precision of model-based estimates (Bossart and Prowell, 1998) and raise mathematical and statistical problems (Whitlock and McCauley, 1999). Small value of F_{ST} cannot be precisely estimated because they correspond to differences in population allele frequencies that are small relative to differences that arise by chance in samples of populations. Because of the non-linearity of the relationship between F_{ST} and N_em, when F_{ST} is small the variance in its estimator is transformed into a much larger variance in the estimator of N_em (Waples, 1998). Neigel (2002) argue that direct estimation based on mark-and recapture or genetic identification of migrant sources is the preferred (*e.g.*, Palsboll *et al*., 1997). Moreover, he mentioned that, although gene flow should be estimated by more powerful approaches whenever practical, F_{ST} remains a useful measure of the average effects of gene flow and will continue to be used for comparative purposes (Neigel, 2002). For avoid such problems, we showed not only N_em but also F_{ST} in this study.

In this paper, we measured local (two or three stations in a region) and regional (Okinawa, Kerama and Ishigaki) patterns of genetic variation and gene flow in *G. aspera* in the Ryukyu Archipelago, southern Japan, using allozyme electrophoresis. We assessed the relative contribution of sexual and asexual reproduction to recruitment. We also tested the hypothesis that gene flow is determined by their planulae competency in a comparison with other species. The reproductive mode of *G. aspera* in the Ryukyus is unique, since other scleractinian corals reproduce only by planula brooding or gamete spawning (reviewed by Harrison and Wallace, 1990). Therefore, a comparison of population genetic data between *G. aspera* and other corals in the Ryukyus provides further information to the relationship between reproductive mode and gene flow in corals.

MATERIALS AND METHODS

Collection and storage of specimens

We established three geographic sampling regions (Okinawa Islands, Kerama Islands and Ishigaki Island), and collected coral samples from two or three reefs that were more than 5 km apart within each region (Fig. 1). Fragments were taken from 38 to 50 colonies at least 3 m apart, which were haphazardly selected at each reef. We kept the collected fragments alive in a small plastic tank filled with seawater, and brought them to the laboratory at the Tropical Biosphere Research Center (TBRC) at the University of the Ryukyus. The fragments were alive after transportation and were reared in an outdoor holding tank (2.0×1.0×3.5 m) supplied with running seawater. The coral fragments were stored at –80°C for at least 1 hr before electrophoresis.

Electrophoresis

Electrophoresis was used to detect variation at polymorphic loci following the methods of Selander *et al*. (1971) and Hillis *et al*. (1996), using horizontal starch gels (11–12%; at 100–180 V and 36–48 mA for 6–9 hr). Five enzyme systems were used: malate dehydrogenase (*Mdh*; E.C.#1.1.1.37), phosphoglucomutase (*Pgm*; E.C.#5.4.2.2), hexokinase (*Hk*; E.C.#2.7.1.1), and peptidase using leucyl-tyrosine (*Lt*; E.C.#3.4.11) and leucyl-glycyl-glycine (*Lgg*; E.C.#3.4.11) as the substrate. Tris EDTA citrate (pH 7.5) buffer was used for *Mdh*, *Pgm*, and *Hk*, and Tris citrate (pH 8.0) buffer was used for *Lgg* and *Lt*.

Statistical analysis

The allele frequencies, measures of genetic variability, and genetic differentiation among and within regions were analyzed using the computer program TFPGA (Miller, 1997). We also assessed the magnitude and direction of departures from Hardy-Weinberg equilibrium (HWE). These departures were expressed as Wright's (1978) fixation index f, where positive and negative values represent deficits or excesses of heterozygotes, respectively. A total of five tests were conducted and Bonferroni's adjustment was used to test the significance of departures from HWE (significant: $P<0.01$).

Two measures were used to assess the possible effects of

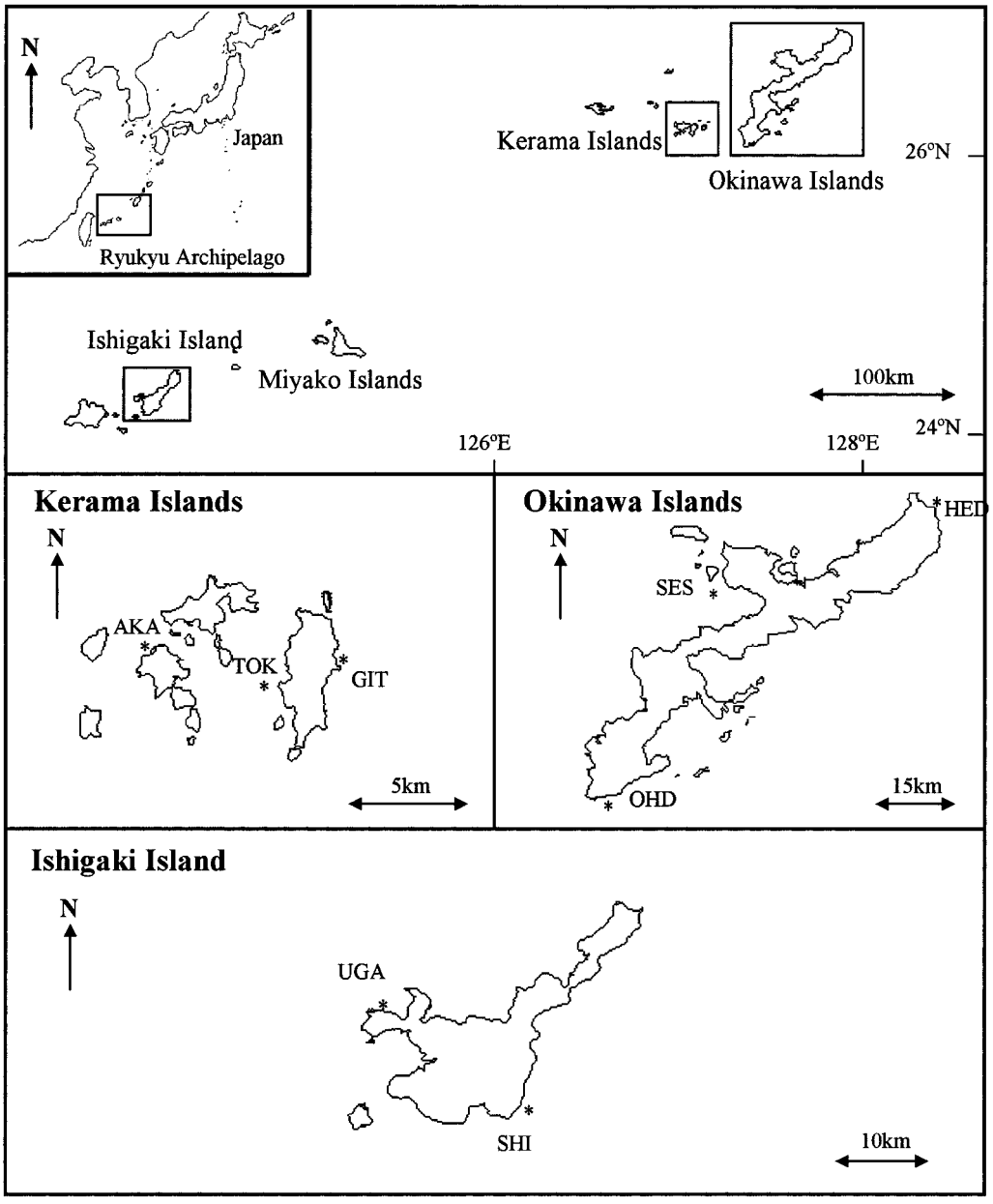

Fig. 1. Map of the Ryukyu Archipelago showing three study regions with detailed collection sites indicated (•). Okinawa Islands. (HED: Hedo, SES: Sesoko, OHD: Ohdo), Kerama Islands. (GIT: Gitsu, TOK: Tokashiku, AKA: Aka), Ishigaki Island (UGA: Uganzaki, SHI: Shiraho).

Table 1. *Goniastrea aspera*. Genetic variability in eight reefs, respectively. Locality abbreviation as in Fig. 1.

Population	Locality	no. of individuals	Mean no.of alleles per locus	% of polymorphic loci (P95)	Mean heterozygosity (S.D.)	
					Observed	Expected
Uganzaki	UGA	49	2.8	66.7	0.269(0.231)	0.306(0.257)
Shiraho	SHI	38	3.5	83.3	0.242(0.175)	0.363(0.212)
Aka	AKA	50	2.7	66.7	0.350(0.333)	0.347(0.323)
Tokashiku	TOK	50	3.2	66.7	0.210(0.258)	0.393(0.325)
Gitsu	GIT	50	2.7	66.7	0.272(0.293)	0.303(0.313)
Ohdo	OHD	46	3.0	83.3	0.349(0.327)	0.414(0.274)
Sesoko	SES	41	3.2	83.3	0.316(0.315)	0.435(0.293)
Hedo	HED	50	3.3	66.7	0.274(0.231)	0.379(0.288)
Mean			3.1	72.9		

asexually derived recruits on the genotypic diversity of the colonies. First, each colony was assigned a multilocus (clonal) genotype. The number of multilocus genotypes detected (N_G) is an estimate of the minimum number of clones present within a population; and the ratio N_G:N (N is the number of individual colonies collected) provides a simple index of the effect of asexual reproduction on genotypic diversity. Second, the ratio of observed multilocus genotypic diversity (G_O) to that expected under conditions of sexual reproduction (G_E) was calculated following Stoddart and Taylor (1988). Departure of G_O:G_E from unity is an index of the combined effect of departures from single-locus HWE and multilocus linkage disequilibrium. A genetically variable population with high levels of asexual recruitment should have a low ratio of observed to expected genotypic diversity. We also tested for significant differences between G_O and G_E using the *t*-test (Stoddart and Taylor, 1988).

We used Wright's (1969) standardized genetic variance (F_{ST}) to quantify the levels of allelic variation among regions or reefs, thereby inferring the degree of population subdivision. We calculated this parameter as Weir & Cockerham's (1984) θ, using the program TFPGA (Miller, 1997), which executes numerical resampling to estimate the variance at each locus (jackknifing) and the variance across loci (bootstrapping). We also estimated gene flow

Table 2. *Goniastrea aspera*. Allele frequency of eight reefs. Collection sites as in Fig. 1. N, number of individuals.

Locus	Allele	Yaeyama		Kerama			Okinawa		
		UGA	SHI	AKA	TOK	GIT	OHD	SES	HED
*Pgm**									
N		49	38	50	50	49	46	40	50
	100	0.531	0.684	0.290	0.290	0.235	0.402	0.325	0.410
	90	0.061	0.040	0.120	0.130	0.102	0.152	0.163	0.060
	85	0.010	0.013	0.010	0.030	0.214	0.011	0.038	0.020
	75	0.000	0.026	0.060	0.290	0.112	0.065	0.113	0.140
	67	0.000	0.000	0.000	0.020	0.000	0.000	0.050	0.030
	57	0.378	0.184	0.520	0.240	0.337	0.370	0.313	0.340
	53	0.020	0.053	0.000	0.000	0.000	0.000	0.000	0.000
*Mdh**									
N		49	38	50	48	50	45	41	50
	140	0.276	0.224	0.280	0.563	0.260	0.400	0.658	0.670
	100	0.714	0.776	0.720	0.437	0.740	0.600	0.342	0.330
	80	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*Hk**									
N		49	38	50	50	50	46	41	50
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Lt-1**									
N		49	38	50	50	50	46	41	49
	110	0.071	0.040	0.160	0.200	0.070	0.043	0.207	0.194
	105	0.071	0.197	0.000	0.020	0.000	0.098	0.000	0.051
	100	0.857	0.684	0.840	0.780	0.930	0.859	0.793	0.745
	95	0.000	0.079	0.000	0.000	0.000	0.000	0.000	0.010
*Lt-2**									
N		44	36	50	50	50	45	40	50
	112	0.000	0.028	0.000	0.000	0.000	0.000	0.025	0.000
	100	0.989	0.847	1.000	0.980	1.000	0.778	0.812	0.960
	93	0.011	0.125	0.000	0.020	0.000	0.222	0.163	0.040
*Lgg**									
N		49	38	50	47	50	44	41	48
	167	0.000	0.026	0.120	0.011	0.020	0.080	0.207	0.073
	142	0.194	0.224	0.210	0.255	0.090	0.420	0.207	0.500
	125	0.224	0.158	0.230	0.340	0.060	0.136	0.281	0.188
	100	0.582	0.579	0.280	0.309	0.640	0.205	0.256	0.229
	78	0.000	0.013	0.160	0.085	0.190	0.159	0.049	0.010

using Wright's (1969) island model (N_em=[(1/θ)–1]/4, where N_e is the effective population size and m is the proportion of migrants per generation).

RESULTS

For the five staining systems surveyed, five polymorphic loci and one monomorphic locus were encoded for *Goniastrea aspera* (Table 1). The mean number of alleles per locus (N_a) in each reef ranged from 2.7 to 3.5, and the percentage of polymorphic loci (P_{95}) ranged from 66.7 to 83.3. The observed mean heterozygosity in each reef was lower than the expected heterozygosity assuming HWE (*t*-test, P<0.01, Table 1).

The allele frequencies of *G. aspera* differed among the three study regions (Table 2). Although *Pgm*53* and *Mdh*80*

Table 3. *Goniastrea aspera*. Estimates of contribution of asexual reproduction in each eight reefs (*N*: number of individuals, N_G: number of multi-locus genotypes, G_o: observed genotypic diversity, G_E: expected genotypic diversity). Locality abbreviations as in Table 1. (unpaired *t*-test, *p<0.05, **p<0.01)

Locality	*N*	N_G	N_G:*N*	G_o	G_E	G_o:G_E
UGA	49	40	0.82	32.89	40.06	0.82
SHI	38	32	0.84	25.79	37.71	0.68*
AKA	50	47	0.94	44.64	49.50	0.90
TOK	50	44	0.88	39.06	50.26	0.78*
GIT	50	41	0.82	34.72	48.33	0.72**
OHD	46	44	0.96	42.32	45.95	0.92
SES	41	41	1.00	41.00	40.96	1.00
HED	50	46	0.92	43.10	49.75	0.87
mean	46.75	41.88	0.90	37.94	45.32	0.84

Table 4. *Goniastrea aspera*. Wright's fixation index (*f*) indicating heterozygote excess (negative number) or deficit (positive number) for each locus in reefs. Asterisks show significant differences among reef after Bonfferoni adjustment for multiple comparisons. (siginificant level: * p<0.05, ** p<0.01, *** p<0.001), (n: no data available).

Locality	*Pgm*	*Mdh*	*Hk*	*Lt-1*	*Lt-2*	*Lgg*	all loci
UGA	–0.035	0.211	n	0.040	–0.009	0.253	0.121
SHI	0.092	0.166	n	0.675***	0.477	0.285	0.333
AKA	–0.084	0.008	n	0.107	n	0.006	–0.008
TOK	0.101	0.831***	n	0.431*	1.000	0.584**	0.464
GIT	0.090	–0.040	n	0.539	n	0.115	0.102
OHD	0.097	–0.019	n	0.740***	0.614***	0.088	0.157
SES	0.171	0.132	n	0.629***	0.920***	0.047	0.275
HED	0.306	0.141	n	0.647***	0.042	0.144	0.276
Mean	0.092	0.179	n	0.476	0.493	0.168	0.215

Table5. *Goniastrea aspera*. Genetic differentiation (F_{ST} ± SE) and gene flow (N_em, average number of migration pergeneration) in three hierachical level are estimated in each pair of region. Statistical significance are calculated by 95 and 99% CI. (Significant level: *P<0.05, **P<0.01). (O: Okinawa, K: Kerama, and Y: Yaeyama)

	K vs O		**Y vs O**		**Y vs K**		**Y vs K vs O**	
	F_{ST}	N_em	F_{ST}	N_em	F_{ST}	N_em	F_{ST}	N_em
Goniastrea aspera								
all reefs	0.070±0.009**	3.3	0.078±0.010**	3.0	0.074±0.004**	3.1	0.078±0.007**	3.0
reefs - region	0.078±0.011**	2.9	0.104±0.014**	2.2	0.084±0.007**	2.7	0.087±0.007**	2.6
all regions	0.025±0.006*	9.6	0.072±0.012*	3.2	0.027±0.010	9.2	0.039±0.004**	6.2
(This study)								
Acropora tenuis								
all reefs	0.022±0.009	11.1	0.042±0.010*	5.7	0.057±0.008**	4.1	0.048±0.008**	5.0
reefs - region	0.026±0.010	9.4	0.051± 0.012	4.7	0.075±0.010**	3.1	0.066±0.009**	3.5
all regions	0.011±0.004	22.5	0.026±0.011*	9.4	0.057±0.008**	4.1	0.015±0.008	16.4
(Nishikawa *et al*., in press)								
Stylophora pistillata								
all reefs	0.128±0.020**	1.7	0.284±0.073**	0.6	0.151±0.039	1.4	0.189±0.042**	1.1
reefs - region	0.148±0.024**	1.4	0.346±0.099**	0.5	0.210±0.055*	0.9	0.215±0.050**	0.9
all regions	0.070±0.018**	3.3	0.260±0.115*	0.7	0.160±0.048**	1.3	0.142±0.050*	1.5
(Nishikawa *et al*., in press)								

were observed only in the Yaeyama region, *Pgm*67* was not observed there. In addition, the frequencies of *Pgm*100* in the Yaeyama region was higher than 0.5, but it ranged from 0.235 to 0.410 in other populations.

The ratio of the number of observed genotypes (N_G) to the number of individuals sampled (N) ranged from 0.82 to 1.00, and the ratio of the observed multilocus genotypic diversity (G_O) to that expected under conditions of sexual reproduction (G_E) among collection sites ranged from 0.68 to 1.00 (Table 3). The ratios N_G:N and G_O:G_E were both high (0.90±0.11 and 0.84±0.07, respectively, mean ± SD). However, the differences between G_O and G_E were significant only for SHI, TOK, and GIT (unpaired *t*-test).

Wright's (1969) fixation index (f) of all loci was positive for all reefs except for AKA in the Kerama region. If the significant deviation from HWE were due to subdivision within populations, one would expect parallel patterns for different loci within each population. However, there is strong pattern of differences among loci, with 5 of 9 significant departures within *Lt-1*. This significant deviation from HWE is likely due to the locus where the electromorphs differed in mobilites least (based on numbering in Table 2). This tendency is in agreement with significant deviations from HWE that were not found at great mobilite locus of *Pgm* (Table 4). In excess and deficits number of heterozygotes, there were almost all deficits (31 out of 38 cases), and significant deviations from HWE were seen in 9 cases.

F_{ST} (genetic differentiation) differed significantly from zero in all pairs of regions, except for all regions between Yaeyama and Kerama, ranged from 0.025 to 0.104 (P<0.05, Table 5). The estimated gene flow (the number of migrants per generation: N_em) calculated from F_{ST} using Wright's island model (1969) ranged from 2.2 to 9.6 among and within regions. In addition, N_em showed similar patterns among each pair of regions (all regions > all reefs > reefs-region, Table 5).

DISCUSSION

Localized asexual recruitment via asexual reproduction or fragmentation had little effect on maintaining populations of *G. aspera*. In the previous study, Sakai (1997) suggested that brooded planulae are very likely the products of sexual reproduction, because eggs remained in the polyps after spawning and there was no sign of asexual reproduction. Asexual reproduction in many clonal marine invertebrates appear to produce (1) low ratios of both N_G:N and G_O:G_E, and (2) a similar number of heterozygotes excesses and deficits (Stoddart, 1983, 1984a, b; Johnson and Threlfall, 1987; Ayre and Willis, 1988; Burnett *et al*., 1995; Adjeroud and Tsuchiya, 1999; Ayre and Hughes, 2000). Previous studies have demonstrated that both ratios are low in coral populations with asexual recruitment. For example, Stoddart (1984) showed that in *Pocillopora damicornis*, which has asexual planulae, N_G:N and G_O:G_E were 0.40±0.17 (mean ± SD) and 0.27±0.15, respectively, and Ayre & Willis (1988) demonstrated that in *Pavona cactus*, which propagates sexually and asexually (fragmentation), the respective ratios were 0.35±0.23 and 0.35±0.31. We found that in *G. aspera*, both N_G:N and G_O:G_E were relatively high: 0.90±0.07 and 0.84±0.11, respectively. In addition, the heterozygotes deficits from HWE were over four times more than the excess. Although this study paid little attention to time scales that may affect ecological and genetic estimates (see McFadden, 1997), we conclude that asexual recruitment likely contributes little to the maintenance of *G. aspera* populations by Sakai's histological observation and present population genetic data.

This study supported our hypothesis that genetic differentiation and gene flow are greater in *G. aspera* than in the brooding coral *S. pistillata*. The values of F_{ST} and N_em among and within regions of a spawning and brooding coral *G. aspera* were significantly greater than that of a brooding coral *S. pistillata* in the Ryukyu Archipelago (P<0.01 and P<0.01, respectively, Wilcoxon signed-ranks test, Table 5). Although there has been no report on brooded planulae of *G. aspera*, the competency period is higher in planulae originating from spawning of *G. aspera* than in brooded planulae of *S. pistillata* (63–70 days and 51 days, respectively, Nozawa, 2000; Nishikawa *et al*., in press). Generally, gene flow is a powerful cohesive force (Slatkin, 1987); one effective migrant exchanged between subpopulations per generation is sufficient to stop the subpopulations from drifting to fixation (alternative gene expression), although higher levels are required to maintain homogeneity (Allendorf and Phelps, 1981). The differences in the competency periods of the two corals indicate that restricted gene flow is likely in *S. pistillata* as compared to *G. aspera*. Moreover, the percentage of mean settlement rate 1 day after planulae release, based on the mean total settlement rates, is 41.5–60.0 and 71.4% in brooded planulae of *G. aspera* and *S. pistillata*, respectively. These results indicate that settlement near parental colonies is more likely in *S. pistillata* than in *G. aspera*. In order to discuss the influence of these initially settlement rates on gene flow, comparisons with other species are needed (*i.e*., with differential initial settlement rates but similar competency periods) in a future study.

This study did not support our hypothesis that gene flow in *G. aspera* is not greatly different from that in the spawning coral *Acropora tenuis*. Planulae originating from spawning in these two corals have similar competency periods (63–70 and 69 days, respectively, Nozawa, 2000; Nishikawa *et al*., in press). However, the genetic differentiation (F_{ST}) and number of migrants per generation (N_em) among and within regions of the spawning and brooding coral *G. aspera* was significantly lower than that of the spawning coral *A. tenuis* in the Ryukyu Archipelago (P<0.05 and P<0.05, respectively, Wilcoxon signed-ranks test, Table 5). Our results indicate that gene flow in *G. aspera* is relatively restricted as compared to that in the spawning coral *A. tenuis*. One possible explanation for the difference in gene flow between the two is a difference in survival rates of larvae; the mean sur-

vival rate was less than 10% of the initial larvae 35 and 59 days after gamete release in *G. aspera* and *A. tenuis*, respectively (Nozawa, 2000; Nishikawa *et al.*, in press). These results indicate that both the competency period and survival rates are important for gene flow in these corals. This study demonstrated that relatively long distance dispersal is likely in *A. tenuis* as compared to *G. aspera*, owing to the different survival rates.

In conclusion, although there are two possibilities of asexual reproduction in scleractinian corals, i.e. asexual production of planula (*Pocillopora damicornis*; Stoddart, 1983) and the fragmentation (*e.g., Pavona cactus*; Ayre and Willis, 1988), asexual reproduction unlikely contributes to maintain local *G. aspera* populations in the Ryukyu Archipelago. Because there was no sign of asexual production of planula by histological study (Sakai, 1997) and the fragmentation unlikely occur in the small massive coral of *G. aspera* in the Ryukyu. Gene flow in *G. aspera* is intermediate between that of *Acropora tenuis* (spawning coral) and *Stylophora pistillata* (brooding coral). These results for *G. aspera* suggest that gene flow attribute to planulae originating from spawning on distant reefs prevents the accumulation of fixed genetic differences among and within regions in the Ryukyu Archipelago.

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