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RESEARCH ARTICLE

Clonal distribution and spatial genetic structure of the reef-building coral *Galaxea fascicularis*

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Abstract

Genotypic distributions affect the persistence of coral populations, and mapping these distributions is important for population management. Many studies have examined genetic connectivity among sites, but within-site spatial genotypic patterns based on clonal distribution and kinship are poorly understood. Such patterns are an important index for understanding the potential for population recovery at small spatial scales. Here, we studied within-reef spatial genotypic distributions and clonality of a broadcast-spawning coral, *Galaxea fascicularis*, by using mitochondrial DNA (mtDNA) and 15 nuclear microsatellite markers. Specimens were collected at shallow reefs (< 3 m) at four sites in the Ryukyu Archipelago, Japan. Among 289 colonies analyzed, we detected two common mtDNA types (mt-L, 174 colonies; mt-S, 113 colonies) and one rare type (mt-L + , 2 colonies). The proportion of duplicate clonal colonies differed across sites and reef topographies; the maximum distance between clonemates was approximately 120 m. Pairwise kinship among colonies tended to decrease with distance at the ramet level (i.e., including clonal replicates), but not at the genet level. Ramet-level kinship varied among sites rather than between mtDNA types. Genet-level kinship (i.e., excluding clonal replicates) was similar among sites. These results for clonality and kinship suggest that both sexual and asexual reproduction contribute to population recovery after disturbances and maintain genetic diversity in local populations. However, the extent of sexual and asexual reproduction differs across sites. Our results will contribute to more effective management of marine reserves by emphasizing the importance of clonal distributions and genetic kinship at each reef site.

Keywords Clonality · Genotypic diversity · Kinship · Microsatellites · Mitochondrial DNA · Spatial autocorrelation

Introduction

Reef-building corals create and maintain coral reef ecosystems and provide ecosystem services that benefit industries such as tourism and fisheries. However, coral reefs have declined during the last several decades because of anthropogenic global climate change and local disturbances (Glynn 1996; Bellwood et al. 2004; Hoegh-Guldberg et al. 2007). Nearshore shallow reefs are especially important for flood reduction, the risk of which is expected to increase with climate change (Beck et al. 2018), and it is important to study and understand their recovery after disturbances. It is well understood that degraded reefs can recover through larval recruitment from other reefs or self-recruitment within natal reefs via sexual reproduction (Underwood et al. 2007). However, many coral species also reproduce asexually by fragmentation (Highsmith 1982; Aranceta-Garza et al. 2012) or by releasing asexual planulae (Stoddart 1983; Yeoh and Dai 2010; Nakajima et al. 2018). Asexually produced clonal replicates are fit colonizers for their local environments, as they bear the same genetic information as their already well-adapted parental colonies (Baums et al. 2006, 2014).

The prevalence of clonal colonies differs both within and among species, depending on the frequency of asexual reproduction (Baums et al. 2006; Nakajima et al. 2016; Zayasu et al. 2016), and it may impact the fitness of local populations. The presence of clonal colonies will help to

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increase the chance of fertilization, because they are considered genets (groups of genetically identical individuals in a given location that arose via asexual reproduction) with high fecundity. This reproductive trait, along with an optimal population size and density, is related to optimal mating opportunities and is known as the Allee effect (Knowlton 2001). To better understand the potential of population recovery through sexual and asexual reproduction, it is important to study the spatial distribution of kinship and clonality on small spatial scales, such as within reefs.

Corals show considerable inter-species differences in reproductive strategies. For example, shorter larval durations or high rates of clonal replication, or both, can increase kinship within localized populations (Miller and Ayre 2008; Gorospe and Karl 2013). Conservation strategies for a coral reef ecosystem as a whole will be improved by studying greater numbers of coral species. The local spatial genetic distributions of several coral species have been investigated already (e.g., *Acropora palmata*, Baums et al. 2006; *Goniastrea favulus*, Miller and Ayre 2008; *Millepora* cf. *platyphylla*, Dubé et al. 2020; *Orbicella annularis* (formerly *Montastraea annularis*), Foster et al. 2007, 2013; *Orbicella faveolata*, Miller et al. 2018; *Platygyra daedalea*, Miller and Ayre 2008; *Pocillopora damicornis*, Combosch and Vollmer 2011, Gorospe and Karl 2013, Gorospe et al. 2015, Gélin et al. 2017; *Seriatopora hystrix*, Underwood et al. 2007, Maier et al. 2009). We focused on the gonochoristic, scleractinian reef-building coral *Galaxea fascicularis* to investigate kinship and clonal distributions within local populations. *Galaxea fascicularis* is a broadcast-spawning coral, in which males and females release gametes simultaneously during synchronized spawning. Female colonies produce pinkish egg bundles and male colonies produce white bundles of sperm and pseudo-eggs, which are thought to make the bundles buoyant (Hayakawa et al. 2005).

Galaxea fascicularis is distributed mainly in the tropical and subtropical Indo-Pacific Ocean (Veron 2000) and in the Ryukyu Archipelago it is found as at least three mitochondrial DNA (mtDNA) types related to genetic divergence (Nakajima et al 2016; Wepfer et al. 2020a, b). These types are easily distinguished by the varying length of the mtDNA non-coding region between genes *nad*2 and *cyt*b (Watanabe et al. 2005; Nakajima et al. 2015). At many sites, *G. fascicularis* produces abundant clonemates by fragmentation (Nakajima et al. 2015, 2016). Previous studies have analyzed the large-scale population genetic structure of this species among reefs in the Northwest Pacific (Nakajima et al. 2016; Wepfer et al. 2022). However, the genetic background and demographic dynamics of local populations of *Galaxea* within the small spatial scale of a reef have yet to be examined. We examined the distribution patterns of genets on the basis of the clonality and kinship of *G. fascicularis* to identify its spatial genetic structure, and we estimated the contributions of clonality and kinship to local population persistence in the Ryukyu Archipelago. We relied on a population genetic procedure using microsatellites, which can be used to identify multilocus genotypes, because clonality and kinship cannot be estimated from simple observations of colonies and reefs.

Materials and methods

Collection of coral samples and genomic DNA extraction

Branches of *G. fascicularis* colonies were collected at four sites: Kuninao (Amami Island), Zampa (Okinawa Island), Ueno (Miyako Island), and Nakano (Iriomote Island) in the Ryukyu Archipelago, Japan (Fig. 1). We collected samples haphazardly while reef walking at Zampa and Nakano, reef walking and snorkeling at Ueno, and snorkeling at Kuninao. When the colonies were next to each other, we collected only one sample from the colonies. All reefs were shallow, with a maximum depth of approximately 3 m, at Kuninao. Zampa, Ueno, and Nakano are flat reefs, but Ueno features a more complex topography with flat, shallow areas and slightly deeper basins (Fig. 1). The collection site at Kuninao is close to the shore, but the seafloor is sloped rather than flat (Fig. 1). The substrate at these four locations is composed of loose rubble. Coordinates for each colony were obtained by using GPS. We collected one or a few branches from each colony. However, one small colony at Ueno, which appeared to have originated from a single polyp that had budded and formed a new colony (Fig. 2), was collected in its entirety. We collected 30 colonies at Kuninao, 99 at Zampa, 51 at Ueno, and 109 at Nakano (a total of 289 colonies at four sites) for subsequent analysis. Collected samples were preserved in 99.5% ethanol and transferred to the laboratory. Samples were preserved at room temperature before extraction of genomic DNA. Genomic DNA was extracted by using a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany).

Genotyping corals with the mtDNA non-coding region

Identification of mtDNA types was performed as described in our previous protocol (Nakajima et al. 2015, 2016) and was based on analysis of the non-coding region between the mtDNA genes *nad*2 and *cyt*b. We used the tailed primer method with PCR to analyze polymorphisms and amplifications of designed primer sets and to identify mtDNA types. The reaction mixture (10 µL) contained template DNA (< 100 ng), AmpliTaq Gold 360 Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), and three primers: a

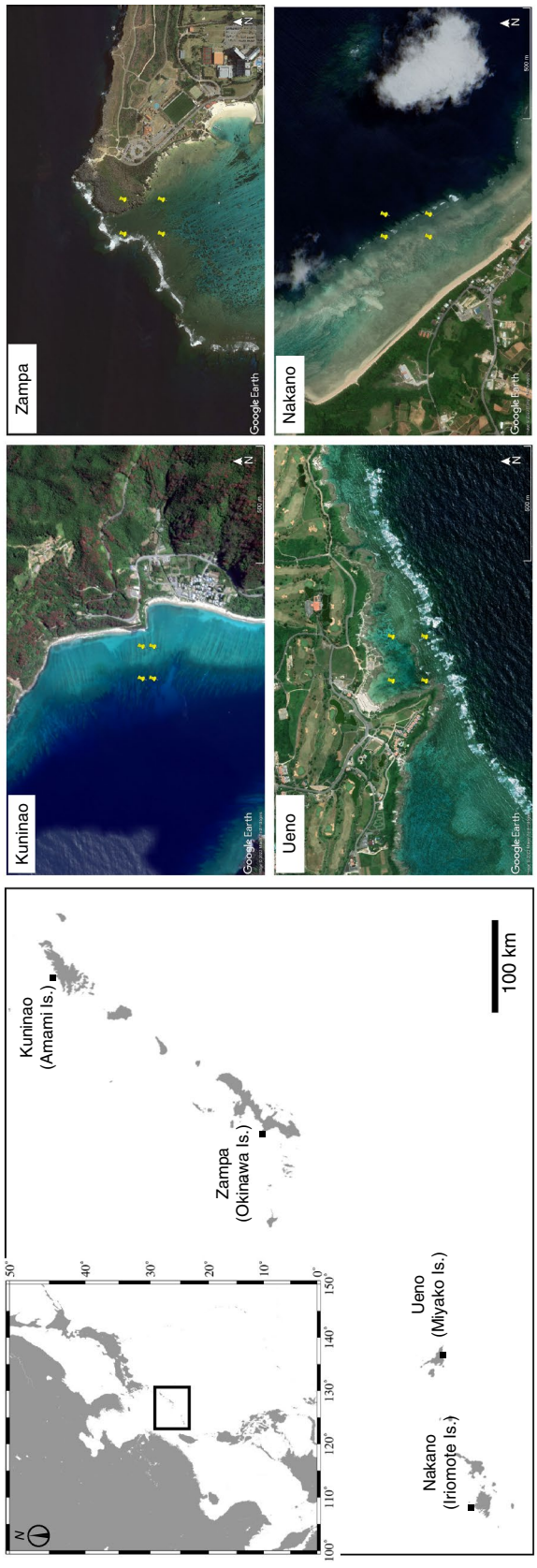

Fig. 1 Map and satellite images of collection sites for *Galaxea fascicularis* in the Ryukyu Archipelago, Japan. Satellite images were downloaded from Google Earth Pro. Yellow pins mark the range of collection points shown in Fig. S1

Fig. 2 Top and bottom views of a small colony of *Galaxea fascicularis* grown from a fragmented skeleton derived from a single polyp collected at Ueno, Miyako Island. An analysis of microsatellite loci showed that the fragmented skeleton and small colony had the same multilocus genotypes. Scale bars are 1 cm

non-tailed forward primer (188–1), a reverse primer with a U19 sequence tail (188-R3-U19), and a tagged primer (U19) fluorescently labeled with VIC dye, based on the method of Schuelke (2000). The primer sequences used for PCR have been provided previously (Nakajima et al. 2016). The final concentration of each primer was 0.2 µM. Amplification was performed with the following PCR conditions: 95 °C for 9 min, followed by 35 cycles of 95 °C for 30 s, 54 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 5 min. Amplified PCR products with internal size standards (GeneScan 600 LIZ; Thermo Fisher Scientific) were analyzed by fragment analysis using an automated capillary-based DNA sequencer (ABI 3130xl Genetic Analyzer, Thermo Fisher Scientific). Fragment peaks were visualized by using Geneious ver. 9.0.4 (Biomatters, Auckland, New Zealand), and fragment sizes were scored manually.

Nuclear microsatellite markers and development of additional markers

We employed eight of the 11 microsatellites previously developed by Nakajima et al. (2015). The remaining three could not be used because they frequently showed a 1-bp shift among some alleles, complicating genotyping (Nakajima et al. 2016). In addition, we developed new microsatellites based on *G. fascicularis* mt-L genomic data described by Nakajima et al. (2015). Ninety-six primer pairs were selected for designing microsatellite primer pairs (4-mer and 10 repeats, 49 loci; 5-mer and 8 repeats, 47 loci) based on the *G. fascicularis* mt-L genome used previously (Nakajima et al. 2015). By using the 96 primer pairs, we successfully amplified 24 loci using mt-L colonies collected at Zampa during a previous study (Nakajima et al. 2015). Of these 24 loci, 13 were successfully amplified in our study. The 13 loci were identified as polymorphic microsatellite markers that were cross-amplifiable for all mtDNA types. Six of the 13 loci were not adequately scored in multiple clonal replicates because of errors derived from a null allele and the occurrence of a nonspecific peak. The remaining seven loci were used for this study as novel microsatellite loci, enabling us to identify clonemates. Characteristics of these seven novel loci and the eight previously reported are shown in Table S1. We thus employed a total of 15 loci for population genetic analysis.

Genotyping corals with the microsatellites

We conducted PCR by using primers for microsatellite loci developed from *G. fascicularis* type mt-L, which are available for both types mt-L and mt-S (see Results and Nakajima et al. 2015). The reaction mixture (10 µL) contained template DNA (< 100 ng), 2 × Multiplex PCR Master Mix (Qiagen), and three primers for each locus: a non-tailed forward primer, a reverse primer with a U19, M13R, T7, or SP6 sequence tail, and a tag primer fluorescently labeled with FAM, VIC, NED, or PET, respectively (FAM-U19, VIC-M13R, NED-T7, or PET-SP6). The final concentration of each primer was 0.02 µM. The combinations of loci and tail sequences for multiplex PCR are shown in Table S1. Amplification of all microsatellite loci was performed with the following PCR conditions: 95 °C for 15 min, followed by 30 cycles of 94 °C for 30 s, 57 °C for 90 s, and 72 °C for 1 min, with a final extension at 60 °C for 30 min. Amplified PCR products were analyzed in the same way as for the identification of mtDNA type, described above.

Detection of clonal replicates

Multilocus lineages (MLLs) were employed to classify clonal replicates (Arnaud-Haond et al. 2007) by using

RClone (Bailleul et al. 2016) implemented in R ver. 3.4.4 (R Core Team 2016). This was done to avoid substandard genotyping due to somatic mutations or scoring errors (Nakajima et al. 2016). We removed one or two loci for estimation of clonality and calculation of the probability of finding identical genotypes resulting from sexual reproduction by chance (P_{SEX}) if a somatic mutation or a scoring error appeared in the target MLL. To remove duplicate clonemates for genet-level analysis, identical multilocus genotypes at a given site were considered clonal colonies ($P_{SEX} < 0.01$). Clonal diversity was estimated with the following index from Dorken and Eckert (2001): $R = (N_{MLL}-1)/(N-1)$, where N_{MLL} is the number of multilocus lineages and N is the number of colonies analyzed. We removed clonal replicates from the dataset, and MLLs were retained for subsequent analyses.

Estimation of population genetic indices

Mean values of observed and expected heterozygosity (H_O and H_E, respectively) and mean values of the fixation index (F_{IS}) across all microsatellite loci at each site were calculated with GenAlEx ver. 6.503 (Peakall and Smouse 2012). Additionally, the significance of F_{IS} across all microsatellite loci for deviation from Hardy–Weinberg equilibrium (HWE) was tested on the basis of 2400 randomizations (Weir and Cockerham 1984) by using FSTAT ver. 2.9.3.2 (Goudet 1995). We calculated the probability of identity (P_{ID}) by using GenAlEx to confirm the resolution of microsatellites.

Kinship between colonies at genet and ramet levels

We used SPAGeDi ver. 1.5d (Hardy and Vekemans 2002) to assess individual statistical correlations between genetic kinship (F_{ij}) (Loiselle et al. 1995) and pairwise spatial distances for all pairs of colonies collected at each site. F_{ij} values were plotted for a range of distance classes, with each class composed of similar pairwise spatial distances between colonies. The number of distance classes was generally set to 10. However, the number of distance classes was set to 5 if the number of pairs in any class would be < 10 if 10 size classes were used. Significance testing was performed for pairwise comparisons between colonies with 10,000 random permutations. We calculated F_{ij} values at both the ramet (with clonal replicates) and genet (without clonal replicates) levels. We used the average latitude and longitude of clonemates for genet-level analysis (Alberto et al. 2005), which assumed the central coordinates of a given genet as the birthplace of the clones. We estimated the *Sp* statistic and the absolute value of the *Sp* reflects the rate of decrease of pairwise kinship with distance and is a metric of the intensity of spatial genetic structure by using the equation $Sp = -blog/(1-F_{[1]})$ (Vekemans and Hardy 2004; Alberto et al. 2005). The slope of a linear regression of kinship coefficient against the logarithm of geographic distance (*blog*) depends on the sampling scale at each location. $F_{[1]}$ is the mean kinship coefficient across loci between colonies or genotypes in the first distance class. These parameters (*blog* and $F_{[1]}$) were also calculated by using SPAGeDi. We calculated the F_{ij} values for each distance class (every 20 m), and therefore the first distance class $F_{[1]}$ was 0 to 20 m. The longest distance between colonies in a location was over 200 m (Nakano), so the 10 distance classes were divided into approximately 20 m each. These distances were then applied to other locations to standardize the distances among locations. For the first distance class in the genet-level analysis for mt-S at Zampa, however, $F_{[1]}$ was calculated for 0 to 40 m to avoid the occurrence of a low number of pairwise samples (only one for 0 to 20 m). The significance of *blog* and $F_{[1]}$ was tested with 10,000 random permutations. The parameter Nb ($=4\pi\sigma^2 D$) is the neighborhood size for the number of breeding individuals in a local neighborhood, based on the concept of Wright (Wright 1946); σ^2 is defined as the variance of the parent–offspring dispersal distance and D is the effective population density (Shirk and Cushman 2014). *Nb* is a parameter of balance between local genetic drift and gene dispersal, and a larger *Nb* means weaker spatial genetic structure within populations (Vekemans and Hardy 2004). *Nb* is the reciprocal of *Sp* and cannot be calculated when $Sp < 0$ without decreasing the pairwise kinship.

Results

Number and distribution of colonies of each mtDNA type

We collected and mapped 289 coral colonies from the four sites. We classified specimens of *G. fascicularis* into mt-L, mt-S, and mt-L + types, with genetic divergence identified by their mtDNA non-coding regions. The proportions of mtDNA types differed at each site (Table 1), even though we collected colonies haphazardly. Of 109 colonies collected at Nakano, most were type mt-L (mt-L, 100 colonies; mt-S, 9). Of the 99 colonies collected at Zampa, 34 were mt-L and 65 were mt-S. We collected two mt-L + colonies, both at Ueno. Both mt-L and mt-S colonies were found at all sites, and they largely overlapped at Kuninao, Zampa, and Nakano but barely overlapped at Ueno (Fig. S1a).

Clonal multilocus lineages and their spatial distributions

Among the 289 colonies collected at our four sites, the genotyping error rate at each microsatellite locus ranged from 0 to 19.72% at the ramet level for all colonies and from 0 to 12.90% at the genet level after the removal of clonal

Table 1 The number and frequency of mtDNA types at each site, and the number of microsatellite multilocus lineages and clonal diversity at each site

Site	mtDNA type	N	N_{MLL}	R	H_O	H_E	F_{IS}	P_{ID}
Kuninao	mt-L	18	18	1.00	0.695	0.808	0.141	2.2×10^{-22}
	mt-S	12	12	1.00	0.566	0.754	0.209	7.5×10^{-19}
Zampa	mt-L	34	14	0.39	0.628	0.797	0.222	5.0×10^{-20}
	mt-S	65	8	0.11	0.562	0.695	0.154	3.1×10^{-15}
Ueno	mt-L	22	8	0.33	0.723	0.822	0.138	3.5×10^{-21}
	mt-S	27	9	0.31	0.474	0.726	0.302	3.4×10^{-17}
	mt-L +	2	2	1.00	–	–	–	–
Nakano	mt-L	100	17	0.16	0.694	0.820	0.145	1.6×10^{-21}
	mt-S	9	5	0.50	0.492	0.632	0.270	1.1×10^{-13}

N is the number of colonies analyzed. N_{MLL} is the number of multilocus lineages (genets), considering scoring errors and somatic mutations (Arnaud-Haond et al. 2007). Clonal diversity was calculated as $R=(N_{MLL}-1)/(N-1)$, in accordance with Dorken and Eckert (2001), for each mtDNA type or site. H_O and H_E are the mean values of observed and expected heterozygosity, respectively. F_{IS} is the index of deviation from Hardy–Weinberg equilibrium. P_{ID} is the probability of identity determined across 15 microsatellite loci. H_O, H_E, F_{IS}, and P_{ID} were calculated on the basis of multilocus lineages at the genet level

replicates (Table S2). There were 93 genets after the deletion of clonal replicates (Table 2). There were no identical multilocus microsatellite genotypes between mtDNA types. Clonal replicates within mtDNA types were detected at Zampa, Ueno, and Nakano, but no clonal colonies were collected at Kuninao for either mt-L or mt-S (Table 1). At the three sites other than Kuninao, clonal diversity R ranged from 0.16 to 0.39 for mt-L and from 0.11 to 0.50 for mt-S. Ten multilocus genotypes accounted for 50.2% of all colonies. The maximum distance between clonemates among all collected colonies was 120 m (mt-S at Zampa; Figs. 3 and S1b).

Heterozygosity and deviation from Hardy–Weinberg equilibrium

Observed heterozygosity ranged from 0.628 at Zampa to 0.723 at Ueno for mt-L and from 0.474 at Ueno to 0.566 at Kuninao for mt-S; the expected heterozygosity ranged from 0.797 at Zampa to 0.822 at Ueno for mt-L and from 0.632 at Nakano to 0.754 at Kuninao for mt-S (Table 1). Total F_{IS} values across all loci ranged from 0.138 at Ueno to 0.222 at Zampa for mt-L and from 0.154 at Zampa to 0.302 at Ueno for mt-S (Table 1). These positive F_{IS} values indicate a significant deficit of observed heterozygosity from HWE at all sites ($Ps<0.0004$). P_{ID} values ranged from 1.1×10^{-13} for mt-S at Nakano to 2.2×10^{-22} for mt-L at Kuninao after clonemate removal (Table 1). The low P_{ID} values confirm that our microsatellites can be used to identify colonies of different lineages. We did not calculate these indices for mt-L + because of the paucity of samples ($N=2$).

Spatial genetic structure by autocorrelation analysis

We determined the extent of kinship among colonies by using the kinship coefficient (F_{ij}) from SPAGeDi (Fig. 4). We used five distance classes (distance intervals for the kinship coefficient) for mt-S at Kuninao and Nakano because of the low number of pairwise colonies (see Materials and Methods). At the three sites where clonal colonies were detected, kinship tended to be highest among proximate colonies for both mt-L and mt-S at the ramet level. We detected high kinship at Ueno and Nakano, and low kinship at Zampa, even between adjacent colonies. Kinship was near zero at Kuninao, with no clonal replicates identified in sampling. At the genet level, we set five distance classes

Table 2 Numbers of colonies in each multilocus lineage identified at each site. Data from Kuninao are not included because there were no clonal replicates of either the mt-L type or the mt-S mtDNA type

Site	mtDNA type	N	Multilocus lineage																
			A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Zampa	mt-L	34	11	4	3	2	2	2	2	2	1	1	1	1	1	1	–	–	–
	mt-S	65	24	22	7	5	4	1	1	1	–	–	–	–	–	–	–	–	–
Ueno	mt-L	22	13	2	2	1	1	1	1	1	–	–	–	–	–	–	–	–	–
	mt-S	27	16	3	2	1	1	1	1	1	1	–	–	–	–	–	–	–	–
	mt-L +	2	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nakano	mt-L	100	39	10	10	9	6	5	4	4	4	2	1	1	1	1	1	1	1
	mt-S	9	5	1	1	1	1	–	–	–	–	–	–	–	–	–	–	–	–

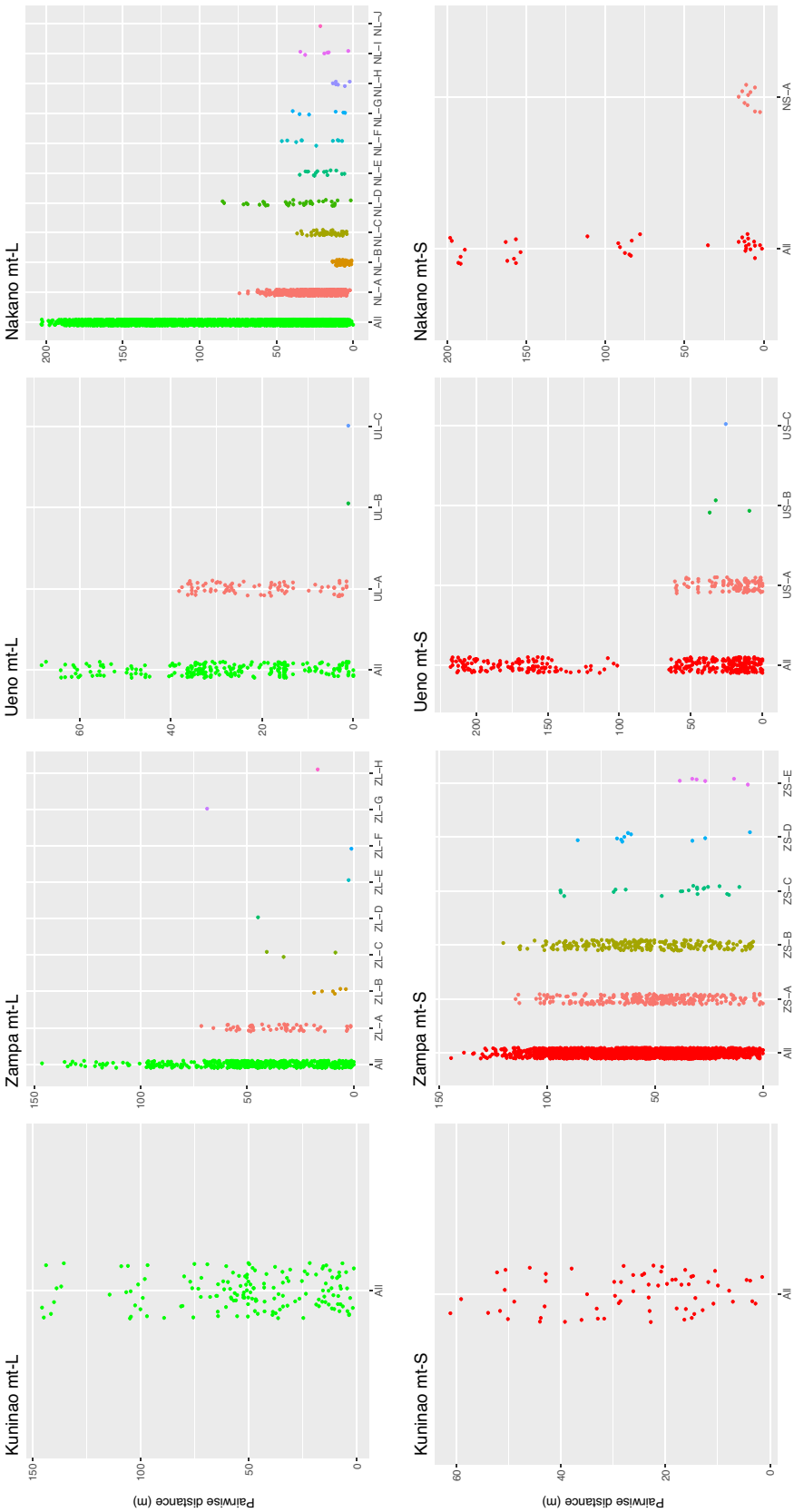

Fig. 3 Pairwise spatial distances of all colonies and multilocus lineages. Red circles, all colonies for mt-L; green, all colonies for mt-S; other colors, specific multilocus lineages at each site. No multilocus lineages are shown for Kuninao because no clonemates were identified at this site. Pairwise spatial distances were calculated with SPAGeDi (Hardy and Vekemans 2002) on the basis of the latitude and longitude of each colony

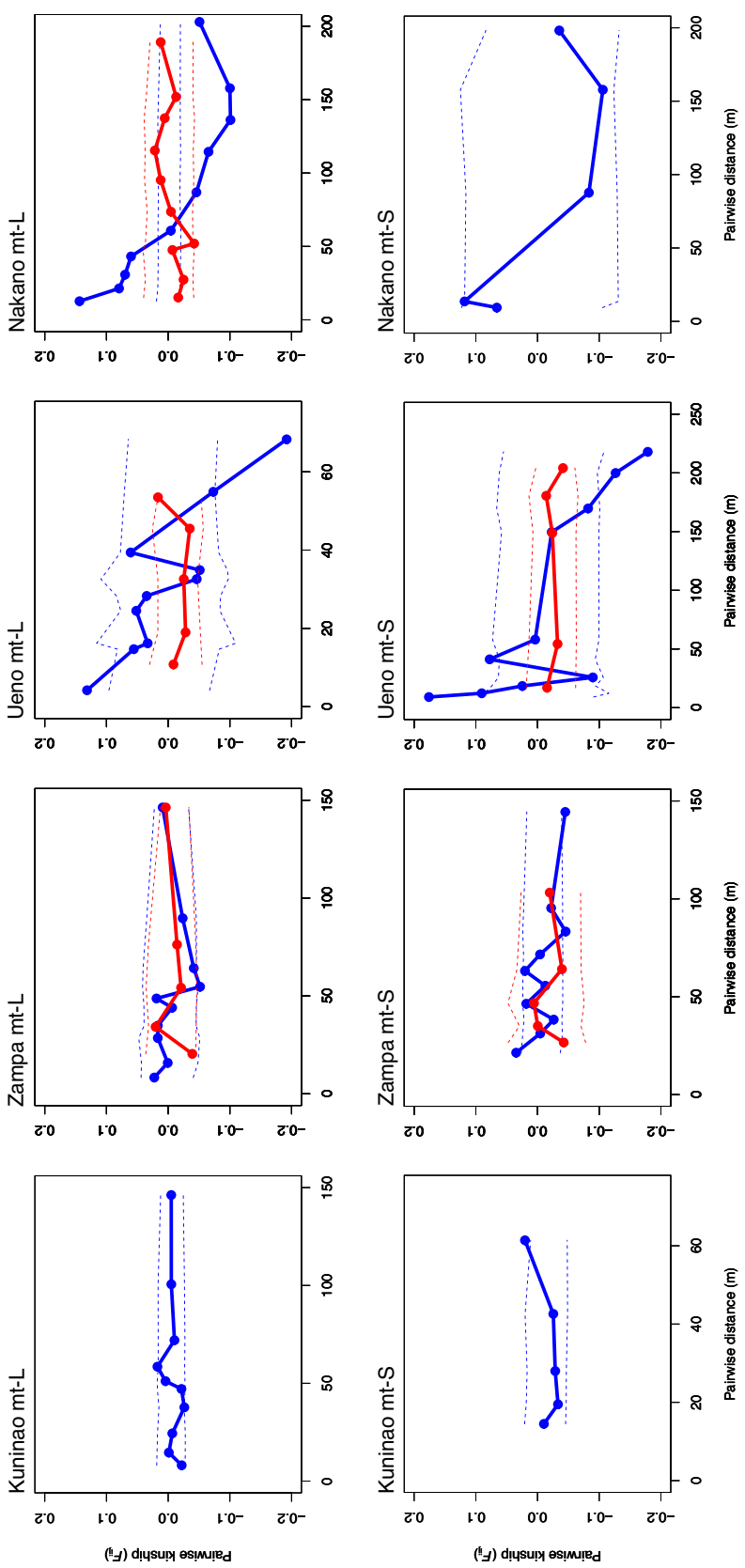

Fig. 4 Kinship between colonies, estimated from F_{ij} values calculated with SPAGeDi (Hardy and Vekemans 2002). Kinship values are shown for all colonies (i.e., at the ramet level; blue) and after removal of clonal replicates (i.e., at the genet level; red). Distances between colonies after removal of clonal replicates were calculated from the means of clonemate latitudes and longitudes. Dashed lines show 95% confidence intervals. The number of distance classes used depended on the number of ramets or genets. No data are shown at the genet level for Kuninao because there were no clonal replicates identified there. The mt-S mtDNA type was not analyzed at Nakano at the genet level because of the small number of genets there ($N_{MLL} = 5$)

for both mt-L and mt-S at Zampa and Ueno because of the low number of pairwise colonies. Also, we did not analyze mt-S sequences at Nakano because of the low number of genets ($N_{MLL} = 5$). At Zampa, Ueno, and Nakano (mt-L only), kinship tended to be lower after removal of clonal replicates. We found statistically significant $F_{[1]}$ and *blog* values ($P < 0.05$) at the ramet level, but on the basis of the *Sp* statistic we detected a weak spatial genetic structure, especially at the genet level ($Sp = -0.001$ to 0.090 at the ramet level and -0.012 to 0.003 at the genet level; $Nb = 11.1$ to 250.0 at the ramet level and 333.3 to 1000.0 at the genet level) (Table 3). *Nb* values could not be calculated at some sites where *Sp* was negative.

Discussion

Both major mtDNA types (mt-L and mt-S) were found at all four sites, but the rare mt-L + type was detected only at Ueno, confirming previous findings (Nakajima et al. 2016). However, the within-reef distribution and prevalence of the types differed among sites; the distributions of the types overlapped at three sites, but not at Ueno. The separation of habitats at Ueno appears to have been influenced by historical recruitment patterns or environmental factors, or both. Ueno features a more complex topography, with flat, shallow areas and slightly deeper basins (see Materials and Methods). On the shallow reef flat, which appears to be strongly exposed to water flow from the reef edge during low tides, mt-S was dominant. Creation of a physical model would help us to understand the physical barriers and local currents and to validate any differences in spatial water flow patterns within Ueno. However, even if the water flow patterns were elucidated, the exact reasons for this separation in the mtDNA types at Ueno would not likely be discovered, because any physiological differences between mt-L and mt-S, including potential differences in stress resistance or mitigation caused by water flow, are unknown. Transcriptome and metabolome analyses will provide valuable insight into this question in the future.

Here, we confirmed the presence of clonal replicates in *G. fascicularis*, as reported in a previous study employing haphazard sampling in which we did not record the coordinates of individual colonies (Nakajima et al. 2016). As a limitation of the data, some of the microsatellite loci showed null alleles or unspecific amplification by our fragment analysis, especially at the ramet level. In future studies, some types of genotyping errors might be resolved through genotyping-by-sequence using next-generation sequencing focusing on short reads.

In this study, the maximum distance between clonal colonies at the genet level was approximately 120 m for mt-S at Zampa, where the maximum distance between clonal replicates was similar for all colonies (approximately 140 m). The maximum distance between genets and between ramets was very similar, suggesting that expanding the sampling region might detect longer-distance clonal replicates. This distance between clones is known to differ among coral species, e.g., 213.28 m for *Millepora* cf. *platyphylla* in Moorea (Dubé et al. 2020), 75.3 m for *Acropora palmata* in Florida

Table 3 Kinship autocorrelation for each site, and mtDNA type based on SPAGeDi (Hardy and Vekemans 2002)

Site	mtDNA type	Input level	$F_{[1]}$	*blog*	*Sp*	*Nb*
Kuninao	mt-L	All (ramet)	− 0.003 ± 0.004	− 0.004 ± 0.003	0.004	250.0
		Genet	− 0.003 ± 0.004	− 0.004 ± 0.003	0.004	250.0
	mt-S	All (ramet)	− 0.022 ± 0.010	0.001 ± 0.007	− 0.001	N.C
		Genet	− 0.022 ± 0.010	0.001 ± 0.007	− 0.001	N.C
Zampa	mt-L	All (ramet)	0.021 ± 0.005	− 0.014 ± 0.003*	0.014	71.4
		Genet	− 0.039 ± 0.017	0.013 ± 0.006	− 0.012	N.C
	mt-S	All (ramet)	0.024 ± 0.007*	− 0.024 ± 0.004**	0.025	40.0
		Genet	− 0.015 ± 0.010	− 0.001 ± 0.017	0.001	1000.0
Ueno	mt-L	All (ramet)	0.072 ± 0.008**	− 0.070 ± 0.009***	0.076	13.2
		Genet	− 0.019 ± 0.015	0.006 ± 0.011	− 0.006	N.C
	mt-S	All (ramet)	0.090 ± 0.015***	− 0.066 ± 0.014***	0.072	13.9
		Genet	− 0.016 ± 0.027	− 0.003 ± 0.013	0.003	333.3
Nakano	mt-L	All (ramet)	0.117 ± 0.011***	− 0.079 ± 0.008***	0.090	11.1
		Genet	− 0.026 ± 0.012	0.010 ± 0.004	− 0.010	N.C
	mt-S	All (ramet)	0.100 ± 0.012*	− 0.052 ± 0.009*	0.057	17.5
		Genet	N.C	N.C	N.C	N.C

Distance classes were defined every 20 m, but the first distance class in the genet-level analysis for mt-S at Zampa was 0–40 m. Some autocorrelation values were not calculated (N.C.). For *Nb*, this was because of the negative value of *Sp*. For mtDNA type mt-S at Nakano, this was because of the small number of genets ($N_{MLL} = 5$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

(Baums et al. 2006), and 13.2 m for *Orbicella annularis* in the Caribbean (Foster et al. 2013). However, these previous studies were from different locations, with different physical and chemical factors such as depth, light intensity, sedimentation, currents, and salinity. In addition, the distances identified are affected by sampling scale and strategy, and therefore a more comprehensive sampling at a standardized scale will be needed for comparisons among species.

Colonies of *G. fascicularis* in shallow habitats appear to be short-lived because of the predominance of small colonies (dozens of centimeters or less) in these areas. Van Woesik et al. (2011) reported that *G. fascicularis* was neither a winner nor a loser after severe bleaching events due to thermal stress in Sesoko, Okinawa, as it is resistant to bleaching (McClanahan et al. 2004). Scattering of fragments after mass bleaching may contribute to recovery during early successional stages (Castrillón-Cifuentes et al. 2017). In *G. fascicularis*, each separated and scattered branch detached from a colony that includes a polyp will bud new polyps from the original polyp (Fig. 2), regardless of season. Meanwhile, the stress-related release of bail-out polyps that settle in the natal habitat has not been confirmed for *G. fascicularis*. Physical disturbances at local or regional scales can split a colony or initiate detachment of polyps or branches. For example, storms appear to promote asexual reproduction via fragmentation (Aranceta-Garza et al. 2012).

The population at Zampa might have a longer history than at the other sites and has nearly achieved equilibrium, because the removal of clonal replicates did little to affect the kinship distributions at Zampa, unlike at Ueno and Nakano. Historical population fluctuations could also influence the number and density of clonal colonies by creating differential fitness among genets (Gorospe and Karl 2013). Genetic diversity based on expected heterozygosity had been maintained even at the three sites with clonal replicates (H_E in mt-L: 0.797–0.822; H_E in mt-S: 0.632–0.726) compared with the values at Kuninao (H_E in mt-L: 0.808; H_E in mt-S: 0.754). In addition, these expected heterozygosities are comparable to the values determined by using microsatellites in *Acropora digitifera* (H_E: 0.569–0.715; Nakajima et al. 2010), which is relatively abundant after mass bleaching events and is known as a winner species in Sesoko (van Woesik et al. 2011). However, these values are higher than the values for *Seriatopora* (H_E in Seriatopora-A: 0.178; H_E in Seriatopora-B: 0.413–0.474; H_E in Seriatopora-C: 0.258; Nakajima et al. 2017), which is a loser species (van Woesik et al. 2011). This means that sexual reproduction might be sufficient to maintain genetic diversity even in these clonal populations, because the expected heterozygosities are comparable to those of *A. digitifera*, a winner species, and the number of genets is sufficient to re-establish populations that are capable of sexual recruitment and genetic exchange, according to the outplanting guidelines of Baums et al. (2019). Moreover, Kuninao is located on a gentle slope with an inconspicuous reef edge, whereas the other three sites are shallower and flat, although the topography at Ueno is complex (Fig. 1).

Wave action on shallow flat reefs may facilitate fragmentation (Castrillón-Cifuentes et al. 2017; Dubé et al. 2017), especially in stormy conditions (Aranceta-Garza et al. 2012). Estimation of the fragmentation rate, physical behavior, and mortality of detached fragments on the seafloor could contribute to a better understanding of the distribution and persistence of clonemates. In addition, elucidating the patterns of water movement would help us to understand the potential for larval retention and inbreeding at a local scale. Future conservation efforts should be informed by analyses of the factors that increase the prevalence of clonemates and the possibility of larval retention at fine spatial scales, including abiotic factors such as hydrodynamics, local climates, and geographic histories, along with the impacts of human activities (Baums et al. 2006; Ulmo-Díaz et al. 2018). Information about genets and clonemates is important to the adaptive potential of outplants for restoration and conservation of coral populations under conditions of successful fertilization, in consideration of anthropogenic climate change (Baums et al. 2019).

Our spatial autocorrelation analysis shows that clonality was linked to spatial autocorrelation and that kinship was randomly distributed at the genet level. F_{ij} values after the removal of clonal replicates resembled those at Kuninao, where no clonal replicates were detected. These findings in the broadcast-spawning species *G. fascicularis* are inconsistent with those of a previous analysis of spatial autocorrelation at tens to hundreds of meters in the brooding species *P. damicornis* at the genet level (Combosch and Vollmer 2011; Gorospe and Karl 2013) and in *S. hystrix* without clonemates (Underwood et al. 2007). As is typical of brooding corals, the dispersal distance of *P. damicornis* larvae is likely to be short (i.e., tens of meters) considering its spatial genetic structure (Combosch and Vollmer 2011).

Larval settlement periods vary widely among species (Richmond 1987; Harii et al. 2002); *G. fascicularis* has a moderately long dispersal period, with larvae settling and metamorphosing 4.5 days after spawning (Babcock and Heyward 1986). Although this is shorter than in *Acropora muricata* (Keshavmurthy et al. 2012), the dispersal duration of *G. fascicularis* is comparable to those of typical spawning corals. This likely helps to maintain the low spatial genetic structuring observed at the genet level within reefs. Furthermore, in *Galaxea*, historical larval dispersal repeated across multiple generations appears to contribute to genetic connectivity among reefs over several hundred kilometers in the Ryukyu Archipelago (Nakajima et al. 2016).

Conclusions

The extent of sexual and asexual reproduction in *G. fascicularis* populations differs across sites. Clonal colonies of *G. fascicularis* strengthen kinship at the ramet level at local scales, but the extent of kinship varies across sites. Larvae originating from sexual reproduction tend to be more mixed within local populations because of the unbiased kinship at the genet level in broadcast-spawning species (Underwood et al. 2009, 2020). This suggests that conservation strategies for marine reserves should consider patterns of clonality and kinship at each reef site to maintain the health of coral populations. Genetic background will influence the adaptive potential for outplanting and the future sustainability of reef organisms (Baums et al. 2019). To generalize our results for spatial genetic structure, further analyses should be conducted in more coral species with different reproductive strategies in the Ryukyu Archipelago.

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Author contributions YN conceived and designed the study. YN and PHW collected the samples. SM contributed reagents and analytical tools. YN performed the laboratory work and analyzed the data. YN wrote the manuscript; PHW and SM helped with the writing. All authors approved the final manuscript.

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Data availability Novel microsatellite loci for *Galaxea fascicularis* were deposited in GenBank (LC507744–LC507750).

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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