



Extremely Stochastic Connectivity of Island Mangroves

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Studies of mangrove population connectivity have focused primarily on global to regional scales and have suggested potential for long-distance connectivity, with archipelagos serving as stepping stones for trans-oceanic dispersal. However, the contribution of propagule dispersal to connectivity is still largely unknown, especially at local-scale. Identifying fine-scale propagule dispersal patterns unique to individual island systems is important to understand their contribution to global species distributions, and to select appropriate sizes and locations for mangrove conservation in archipelagos. Using population genetic methods and a release-recapture method employing GPS drifting buoys, we investigated the spatiotemporal scale of propagule dispersal of *Rhizophora stylosa*, one of the widely distributed mangrove species in the Indo-West Pacific. This study sought to quantify intra- and inter-island connectivity and to assess their contributions to oceanic scale dispersal of *R. stylosa* from the Ryukyu Archipelago, which spans over 545 km in southwestern Japan. Using 7 microsatellite markers, we tested 354 samples collected from 16 fringing populations on 4 islands. We identified 3 genetic populations, indicating distinct genetic structures comprising 3 distinguishable bioregions (genetic clusters). The western end of the archipelago receives relatively frequent migration ($m > 0.1$), but is genetically isolated from other sites. Based on genetic migration rates, we found that the central area of the archipelago serves as a stepping stone for southwestward, but not northeastward dispersal. On the other hand, with *in-situ* drifting buoys, we did not confirm prevailing dispersal directionality within the archipelago, instead confirming local eddies. Some buoys trapped in those eddies demonstrated potential for successful beaching from another island. A large portion of buoys were carried predominantly northeastward by the Kuroshio Current and drifted away from the coastal areas into the Pacific, contrary to local migrations. We found that the spatiotemporal scale of propagule dispersal is limited by the distance between islands ($< 200\text{km}$), propagule viability duration, and fecundity. Over all, recruitment does not occur frequently enough to unify the genetic structure in the archipelago, and the Ryukyu Archipelago is isolated in the center of the global mangrove distribution.

Keywords: mangrove, population connectivity, stepping stones, island system, biophysical approach, propagule dispersal, drifting buoy tracking

INTRODUCTION

The spatiotemporal scale of mangrove propagule dispersal is foundational to understand demographic connectivity among islands that support fragmented, fringing mangrove populations. Demographic connectivity is defined as the relative contribution of propagule dispersal to population growth rate in comparison to local recruitment, and it is an important concept for conservation, especially when changes in propagule translocation result in a negative shift from stable and/or positive population growth (Lowe and Allendorf, 2010). Fragmented mangrove populations are vulnerable to environmental changes such as coastal development, climate change, and sea level rise. Mangrove populations at the edges of their distribution commonly exhibit low genetic diversity suggesting their higher risk to negative effects from monomorphism and inbreeding compared to mangrove habitats in the center of the species range. Although mangrove habitats are protected through international agreements in order to mitigate the impacts of changing climate on coastal ecosystems, conversion and degradation of mangroves are continuing, especially for smaller mangrove patches (Curnick et al., 2019). Complicated and spontaneous mangrove establishment at local- and fine-scales tends to occur in island systems (Triest et al., 2021a), and some islands facilitate global distribution of mangroves by acting as stepping stones for transoceanic dispersal (Van der Stocken et al., 2019). Thus loss of small mangrove habitats on islands may impact global distribution of mangroves in the long run, if not in the near term.

While demographic connectivity is important to sustain population sizes, to colonize unoccupied niches, and to assemble local communities from the meta-community (Levin et al., 2003; Levine and Murrell, 2003), the effects of population loss or degradation on other populations in the same island system are not well understood. Thus, understanding the spatiotemporal scale of propagule dispersal should help estimate demographic connectivity among islands, thereby helping to design effective protected areas.

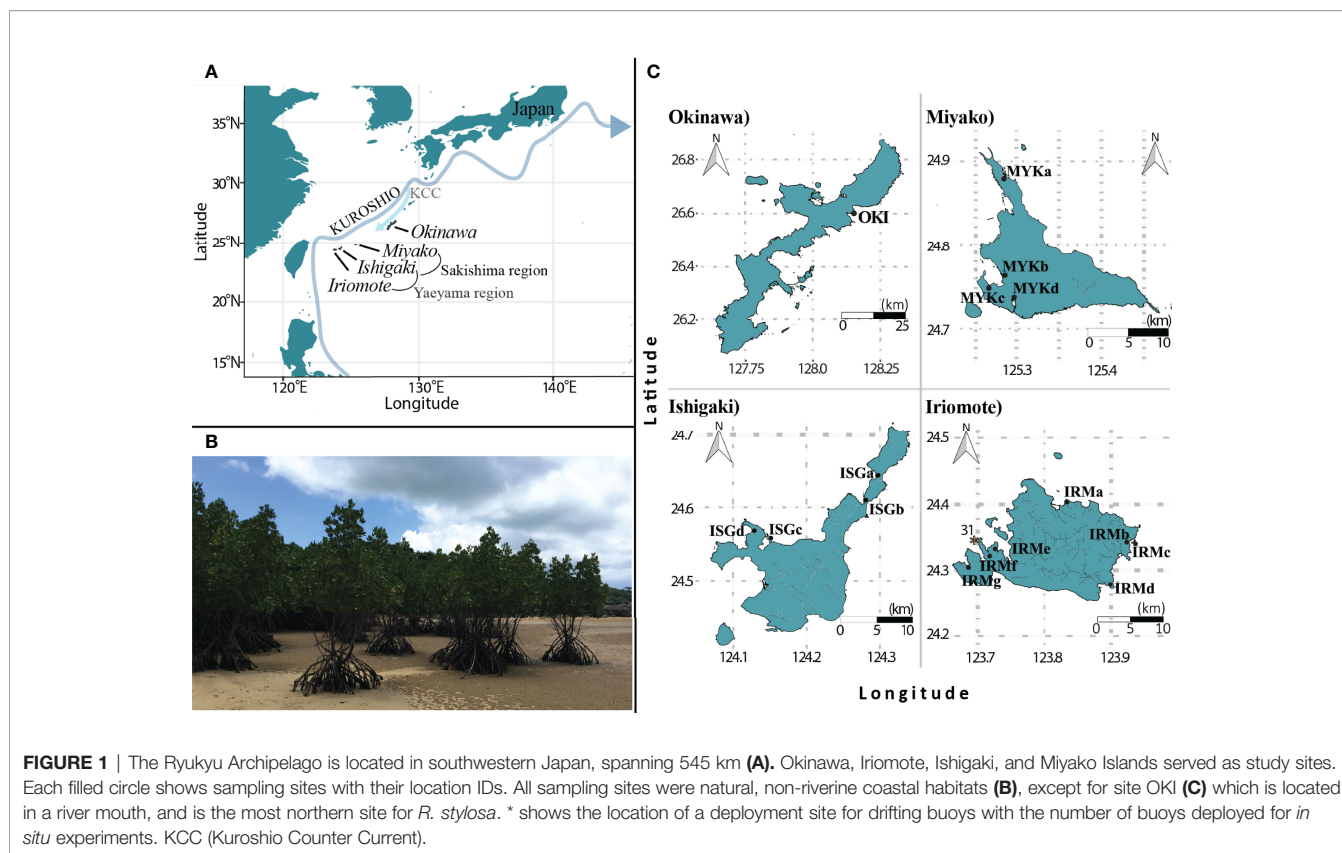
Genetic methods have been used to understand spatial scales of population connectivity. Previous genetic studies have supported transoceanic connectivity, and have identified bioregions indicating genetic discontinuities among distant populations over biogeographic timescales (Dodd et al., 2002; Takayama et al., 2013; Lo et al., 2014). Spatial patterns of genetic discontinuity have been compared with ocean currents, prevailing winds, landscapes, and seascapes to understand mechanisms of dispersal that cause them. For example, the Central American Isthmus was suggested as a land barrier for dispersal of *Rhizophora mangle* and *Rhizophora racemosa* (Takayama et al., 2013), as was the Mexican coast for *Avicenna germinans* and *R. mangle* (Sandoval-Castro et al., 2012). Others have shown that mangrove connectivity is related to oceanic currents, particularly their direction along coastlines, and that migration routes follow major oceanic and coastal currents (Triest et al., 2021a).

On the other hand, mangrove propagule dispersal in island systems has received less attention than continental coastal

dispersal. A few studies on mangrove genetic connectivity in island systems have suggested low genetic variation and limited genetic connectivity (Islam et al., 2012; Islam et al., 2014; Yahya et al., 2014; Al-Qathanin and Alharbi, 2020). Except for Yahya et al. (2014), a study on the Sunda Islands in Indonesia, archipelagos investigated in previous studies were located near the distributional edges of the studied species, where species are subjected to more genetic drift and environmental selection compared to those in central regions (Arnaud-Haond et al., 2006). Those studies also suggest that discreteness of island coastlines is a potential cause of limited genetic variation, regardless of distributional area. However, because of the paucity of studies in island systems, differences in studied species and different studied area sizes, more island studies with various species are needed to understand demographic connectivity in archipelagos.

Although genetic methods can detect the outcome of connectivity interference caused by physical processes such as hydrochory (dispersal *via* water), genetic information alone is insufficient to estimate spatiotemporal scales of connectivity (Lowe and Allendorf, 2010). Knowledge of propagule immigration revealed by genetic data, combined with physical oceanographic information obtained from numerical ocean modeling and/or empirical release-recapture methods, have been used to investigate demographic connectivity (Nathan and Muller-Landau, 2000; Nathan et al., 2008; Ngeve et al., 2017). In the past decade, with advances of computational power and modeling processes, numerical ocean modeling has been extensively incorporated into dispersal and connectivity studies (Fox-Kemper et al., 2019) including mangrove connectivity studies to interpret genetic results at regional and global scales (Pil et al., 2011; Wee et al., 2014; Ngeve et al., 2016; Geng et al., 2021). For example, a previous biophysical approach suggested an oceanic barrier between the Andaman Sea and the Malacca Strait around the Malay Peninsula for dispersal of *Rhizophora mucronata* by interpreting genetic outcomes with simulated ocean currents (Wee et al., 2014). Ngeve et al. (2016) identified an oceanic barrier at an oceanic convergence zone near the Cameroon Estuary Complex for dispersal of *R. racemosa* using a genetic approach with numerical particle tracking models. Van der Stocken et al. (2019) quantified potential for long-distance propagule dispersal at global scale using a Lagrangian particle tracking model. They found along-coast transport dominant over many continental coastlines, as well as stepping-stone dispersal *via* several Pacific island systems (the Galapagos Islands, Polynesia, Micronesia, and Melanesia) that allow for trans-oceanic dispersal. Their findings suggested potential effects of minimum and maximum floating periods of propagules in determining the extent of dispersal. However, detailed spatiotemporal studies of dispersal are not yet understood for island systems.

Therefore, this project was designed to investigate the spatiotemporal scale of propagule transport among islands. The model site was the southern half of the Ryukyu Archipelago (**Figure 1A**), a poleward peripheral region of the studied species, *Rhizophora stylosa* (Rhizophoraceae) in the



northern hemisphere. The total area of mangrove in Japan is about 553 ha, from the Ryukyu Archipelago to Kagoshima Prefecture (31°20'N) of which 80% occurs on Iriomote island (Figure 1C) with 7 - 19 species (Minagawa, 2000). According to radiocarbon studies on one of the largest rivers on Iriomote Island (Urauchi River; 7.45 km), the present mangrove forests were established over 1,000 years ago (Fujimoto et al., 2015). Depending on locations in the archipelago, development of mangrove habitats varies between 400 years BP and 3,500 years BP (Fujimoto and Ohnuki, 1995; Fujimoto et al., 2015), which is relatively recent compared to global mangrove establishment. During the establishment period, the sea level was the highest between 1,700 and 5,100 years BP, and declined to the current sea level in the southern part of the archipelago about 1,000 years BP (Yamano et al., 2019). The archipelago is located 150–200 km to the east of the Kuroshio western boundary current flowing northeastward (Figure 1A). The previous oceanographic study regarding coral larval dispersal around the archipelago showed that most particles are carried southward by the Kuroshio Counter Current (Figure 1A) from Spring to Fall, and eastward transport of particles from the Kuroshio toward the western coast of Okinawa Island occurs primarily in warm seasons (Uchiyama et al., 2018). Additionally, the probability of long-distance dispersal (LDD) maybe largely influenced by oceanic conditions south of the archipelago, indicating complex surface hydrodynamics in the region (Uchiyama et al., 2018).

Sampling sites for the current study were focused on fringing, non-riverine mangrove habitats (Figure 1B). We predicted that non-riverine sites proximal to the open ocean would facilitate island-to-island propagule transport, based on findings of previous studies (Kadoya and Inoue, 2015; Triest et al., 2021a). *Rhizophora stylosa* was chosen as the model species for this study, based on its propagule traits. *Rhizophora stylosa* propagules have higher dispersal capacity than other mangrove species in the Ryukyu Archipelago (e.g. *Bruguiera gymnorhiza* and *Kandelia obovata*), which is determined mainly by positive buoyancy upon detaching from parent trees, the length of the viable period while submerged in water, and root initiation time while floating (Kadoya and Inoue, 2015; Wang et al., 2019). By comparing those dispersal properties, *Rhizophora* appears to have great potential for LDD, especially *R. mangle* and *R. stylosa* because of their long floating periods (over 100 days in the laboratory) (Rabinowitz, 1978). They also have the capacity to tolerate high salinity and dryness (Clarke et al., 2001; Kadoya and Inoue, 2015, respectively), and to maintain a vertical orientation that is favorable for dispersal maneuvering between roots and landscapes of mangrove forests more easily compared to horizontal orientation (Van der Stocken et al., 2015). However, reproduction *via* propagules is significantly lower than those of other tropical and subtropical coastal species, such as seagrass and corals. Fecundity of mangrove species depends on the reproductive stage. Coupland et al. (2006) studied 20 reproductive shoots and studied 222 young buds

from 10 trees. They showed a poor rate of pollination (0.5%), which is accomplished by wind (Yan et al., 2016), as well as a poor conversion rate (< 3%) from flowers to mature propagules, which resulted in an extremely low propagule fertility rate. Such low conversion rate of *R. stylosa* was found in a study on litter in mangrove forests in Okinawa Island as well (Sharma et al., 2011). Producing large propagules, like those of *R. stylosa*, is metabolically expensive; therefore, not only restricted pollination, but also resource limitation interferes with propagule reproduction (Coupland et al., 2006). Hence, the species propagule fecundity appears to be substantially low, however is yet understudied. It has also been argued that the efficiency of wind pollination is low in fragmented habitats (Ngeve et al., 2016). Hence, the primary method for mangrove gene exchange seems to be propagule dispersal.

We used 7 microsatellite markers for 354 samples collected from 16 populations on 4 islands, and obtained spatial information regarding genetic connectivity and migration rates to quantify potential for propagule transport between every site pair over several generations. To determine the temporal scale of dispersal trajectories and to quantify potential for direct dispersal within the archipelago, we used a release-recapture method employing GPS tracking-drifting buoys. These buoys float along the surface current and provide spatiotemporal information about geographic position until they beach or turn off due to battery exhaustion. Using ocean current data combined with genetic data and propagule ecological information, we estimated demographic connectivity among islands with detailed information about propagule dispersal.

MATERIALS AND METHODS

Study Sites and Collection of Mangrove Specimens

Sampling sites included 16 sites on 4 islands spanning 545 km (Figure 1A). The area of individual sites ranged from 0.04 ha to 8 ha, small in comparison with other tropical mangrove populations. All sample sites were natural habitats where no mangrove reforestation had occurred, according to local records. Okinawa Island (Figure 1C) is *R. stylosa*'s most northern habitat (Spalding et al., 2010), and has been protected by the Ministry of the Environment in Japan. Iriomote Island (Figure 1C) has been protected since 1972 as a Japanese National Monument. All sampling sites, except for one riverine habitat in Okinawa Island are fringing habitats with tree heights from 1.5 m to 5m. We collected leaf samples from 20 to 40 trees that were 5 to 10 meters apart in order to avoid sampling overlap. We cut leaves from their petioles, and immediately preserved them in sealable bags with silica gel to dehydrate them. In order to completely dehydrate them within 24 h, we discarded the main veins of leaves and cut the leaves into smaller pieces within 12 h after collection. After samples were brought back to the lab, they were kept in plastic bags with silica gel, sealed in a container, and kept at 25°C until DNA extraction.

DNA Extraction and Microsatellite Analysis

For genomic DNA extraction, 0.03–0.05 g of each leaf was homogenized with zirconia beads using a cell disrupter (Tommy Micro Smash MS-100) at 2,500 rpm for two cycles of 29 s to achieve evenly homogenized specimens. Then, we used a QIAGEN DNeasy Plant Kit to extract DNA, and quantified collected DNA with a NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) before storing it at -20°C until use. We analyzed polymorphisms of isolated loci for 354 samples from 16 populations with 11 nuclear microsatellite markers (ncSSR) of which 8 ncSSR markers (Rhst01, Rhst02, Rhst13, Rhst15, Rhst16, Rhst19, Rhst20, Rhst27) were developed by Islam et al. (2004), and the other 3 ncSSR (RM110, RM107, RM121) were developed by Shinmura et al. (2012). Seven loci (Rhst01, Rhst15, Rhst19, Rhst20, Rhst27, RM107, RM121) were successfully amplified and showed polymorphisms under the following conditions.

Polymerase chain reactions (PCR) were performed in 10- μ L reactions containing 10 ng/ μ L DNA template, QIAGEN Multiplex Master Mix, 0.2 μ M of each primer pair, 0.2 μ M of each fluorescence-labeled probe, and RNase-free H₂O. PCR cycle conditions were 15 min at 94°C, followed by 35 cycles of 30 s at 94°C, 90 s at 60°C annealing temperature, and 60 s at 72°C in the final cycle. For samples that failed to amplify with the method above, each locus was amplified individually. PCR solution in this case was 10 μ L which consisted of 1 μ L DNA template, 5 μ L of QIAGEN AmpliTaq Gold 360 Master Mix, 1.5 μ L MilliQ water, and 0.2 mol primer pair and fluorescent-labeled probe. PCR cycle conditions for AmpliTaq included 5 min at 90°C initially, followed by 35 cycles of 30 s at 95°C, 30 s at 50°C annealing temperature, and 60 s at 72°C in the final cycle with a final extension at 72°C for 10 min. PCR products were then diluted 5x with MilliQ water and applied to a 3130 xl Genetic Analyzer by Applied Biosystems to analyze allelic variation. Fragment sizes were analyzed with Geneious 11.0.3 (Biomatters Ltd., Auckland, New Zealand) relative to internal lane size standards using a GeneScan™ 600 LIZ® (Thermo Fisher Scientific).

Genetic Data Quality Check

Prior to population genetic analysis, we tested the probability of identity (PI) and linkage disequilibrium of genetic data. PI was tested to determine whether any two individuals accidentally shared an identical multilocus genotype, to derive a cumulative probability of identity for all polymorphic loci in each population using GenAlEx 6.5 (Peakall and Smouse, 2012), and gave a total value of 2.9×10^{-4} to 5.4×10^{-1} for all polymorphic loci in each site. Linkage disequilibrium for each locus pair across all populations was tested using Fisher's method on GENEPOP on the web (Raymond and Rousset, 1995; Rousset, 2008), and detected significant linkage disequilibrium ($P < 0.01$) for Rhst15 & Rhst20 (MYKc, ISGa, IRMe), Rhst01 & Rhst15 (IRMa, IRMc), Rhst01 & Rhst19 (MYKd), Rhst15 & Rhst27 (IRMd), Rhst20 & Rhst27 (IRMd), Rhst20 & RM107 (ISGa), and Rhst27 & RM107 (IRMc). In the majority of population, paired loci with significant linkage disequilibrium were not observed.

Genetic Diversity

To analyze genetic diversity, we computed expected heterozygosity (H_e), observed heterozygosity (H_o), and pairwise population genetic differentiation (F_{STP}) using GenAlEx (Peakall and Smouse, 2012). $F_{STP} < 0.05$ was set as the threshold for insignificant genetic differentiation between paired sites (Frankham et al., 2010). The number of alleles (A_r) per locus and per population, and the inbreeding coefficient (F_{IS}) for each population were analyzed using FSTAT 2.9.3 (Goudet, 2001). Allelic richness was rarefied to the smallest population size of 9 (MYKb) based on a rarefaction method (Hurlbert, 1971). To evaluate population differentiation, analysis of molecular variance (AMOVA) was conducted within and among populations using GenAlEx 6.5 (Peakall and Smouse, 2012).

Genetic Structure

To identify genetic structures of individual samples, we used a Bayesian clustering model, STRUCTURE 2.3 (Pritchard et al., 2000; Hubisz et al., 2009). Each run assigned K (1 to 18) clusters to each sample. All parameters were set to default. With a Bayesian Markov chain Monte Carlo (MCMC) resampling method, each run underwent 500,000 iterations, after discarding 80,000 as burn-in for 20 replicates. The optimal K for this dataset was determined with Structure Harvester (Earl and Holdt, 2012) using results from STRUCTURE, and the likelihood of multiple values of K was tested using the Evanno method (Evanno et al., 2005). With CLUMPAK (Kopelman et al., 2015), the entire set of runs obtained from STRUCTURE was merged for each K value, based on similarity matrices, and visualized.

Isolation by Distance

To test the effect of geographic distance on genetic connectivity, we compared pairwise genetic differentiation (F_{STP}) and geographic distance between every population pair using Mantel's test in GenAlEx 6.5 (Peakall and Smouse, 2012). All F_{STP} values were linearized ($\text{Lin}F_{ST} = F_{STP}/1 - F_{STP}$), and we measured the Euclidean distance between populations using Google Earth Pro.

Bayesian Assignment Tests

To identify differences in contemporary patterns of migration (a few generations) from historical patterns of population connectivity, we estimated recent migration rates between site pairs with BayesAss 3.0.4 (Wilson and Rannala, 2003), which uses a MCMC resampling method. BayesAss assumes that migration does not change allelic frequencies over two generations, and that output migration rates reflect the most recent several generations. We computed five replicates with different random starting seeds for an MCMC run with 10,000,000 iterations, discarding the first 1,000,000 as burn-in in each run. Samples were collected every 100 observations with default parameter settings. Sample site MYKb was excluded from this analysis due to its inadequate sample size. We examined posterior mean migration rates (m) for gene flows between sites. We followed (Faubet et al., 2007) for performance analysis of the test.

In Situ Drifting Buoy Experiments

31 drifting buoys were deployed in order to estimate effects of ocean hydrodynamics on propagule dispersal. Drifter deployment was carried out with one drifter per day for 31 consecutive days (except when weather or sea conditions made it unsafe to conduct field work) starting on August 7, 2018, which is the middle of fruiting season on Okinawa Island (Sharma et al., 2011), and a little after its peak in July for Iriomote Island (Kadoya and Inoue, 2015). To also avoid major typhoon season, August was reasonable time of the year for deployment. Buoys were deployed from just off the reef of IRMG on Iriomote Island (* on **Figure 1C**), where clear genetic isolation was detected despite the proximity of neighboring populations (< 5km to the closest population). In order to obtain ocean surface current vector information, we used Microstar drifters developed by Pacific Gyre. Microstar drifters have two parts, a surface float (20 cm in diameter) and an underwater corner-radar-reflector-type drogue (Ohlmann et al., 2005). The float maintains its drogue at a constant depth of 1 m below the surface to capture the surface current, and minimizes the effects of wind and surface waves with its spherical shape. Drogues were calibrated to provide about < 2 cm/s velocity differences from the "tagged" water parcel with little slip measurement under calm wind (< 12 m) (Ohlmann et al., 2005). Direct effects of high wind on Microstar drifters are not yet well understood. Drifter positions were determined by GPS at near-real time, using the Iridium satellite data network with user-specified up-linking intervals, which allowed us to collect spatiotemporal information of dispersal trajectories from all deployed buoys. We set the intervals so that positioning information with higher resolution would be obtained near the coast. Intervals were set from 5 min to 12 h depending on the distance from the nearest coast. Drifters can be positioned with an accuracy of < 7m (Ohlmann et al., 2005). Collected spatiotemporal data were analyzed using visualized using MATLAB.

RESULTS

Genetic Diversity

The average number of alleles (allelic richness) per population varied depending on sites from 1.2 (OKI) to 2.7 (IRMc) (**Table 1**). This study showed little to no heterozygosity (H_o) in any population, and at all loci, and expected heterozygosity (H_e) consistently exceeded H_o for all sampling sites. The inbreeding coefficient (F_{IS}) exceeded 0.8 ($p < 0.01$) for all sites, while pairwise genetic differentiation (F_{STP}) varied depending on sites ranging from 0.017 (IRMe-IRMf) to 0.828 (IRMG-OKI) (**Figure 2**). We found four site pairs (three neighboring pairs, IRMf-IRMe, ISGa-ISGc, MYKb-MYKd and one inter-island pair, IRMd-MYKc) with insignificant pairwise genetic differentiation ($F_{STP} < 0.05$) (**Figure 3**). The hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992) showed 78% of genetic variation occurred among individuals within sites, 12% of variation occurred among sites, and 10% of variation occurred among islands ($p < 0.01$) (**Table 2**).

TABLE 1 | Sampling site information and genetic variability of each site.

Island	Population	Lat	Lon	Area (ha)	Geographical traits	N	Ar	Na	Ho	He	Fis
Okinawa	OKI	26.604	128.143	7.712	River mouth	13	1.21	1.286	0.000	0.022	1.000
Miyako	MYKa	24.789	125.286	8.133	Inlet bay	19	2.00	2.143	0.008	0.389	0.982
	MYKb	24.763	125.282	0.317	Inner bay	9	1.69	1.714	0.000	0.238	1.000
	MYKc	24.752	125.268	0.294	Sandy beach	21	2.50	2.857	0.000	0.437	1.000
	MYKd	24.731	125.296	3.787	Sandy beach/bay	26	2.21	2.571	0.013	0.329	0.962
Ishigaki	ISGa	24.542	124.296	0.096	Sandy beach	27	2.24	3.143	0.005	0.277	1.000
	ISGb	24.510	124.279	0.191	Inlet beach	21	2.36	3.000	0.014	0.399	0.967
	ISGc	24.456	124.149	0.044	Inlet	21	2.03	2.571	0.007	0.275	0.977
	ISGd	24.467	124.125	1.024	Inlet	30	2.58	3.000	0.034	0.452	0.926
Iriomote	IRMa	24.403	123.830	3.688	Sandy tidal flat	26	2.54	3.000	0.000	0.433	1.000
	IRMb*	24.344	123.934	7.218	Sandy tidal flat	18	2.06	2.286	0.008	0.349	.0979
	IRMc*	24.344	123.928	1.776	Rocky tidal flat	28	2.66	3.000	0.005	0.482	0.990
	IRMd*	24.279	123.904	9.560	Inner bay	27	2.53	2.857	0.000	0.477	1.000
	IRMe**	24.334	123.728	2.580	Inner bay	12	2.34	2.571	0.036	0.325	0.899
	IRMF**	24.331	123.714	2.584	Inner bay	30	2.54	3.571	0.058	0.282	0.839
	IRMg**	24.309	123.683	0.206	Inner bay	26	1.40	1.857	0.016	0.091	0.826
Total						354	Avg 2.84	2.589	0.013	0.328	0.959

* Sites are East Coast of Iriomote Island (ECI).

** Sites are West coast of Iriomote Island (WCI).

We found rather high inbreeding coefficients (F_{IS}), and low observed heterozygosity (H_o) at all sites compared to similar island mangrove population connectivity studies, including that of Islam et al. (2014). Results indicate extremely rare gene exchange occurring among sites in the archipelago.

Isolation by Euclidian Distance

Mantel tests revealed that distance accounts for < 5% of genetic differentiation between sites in the Yaeyama region ($R^2 = 0.045$, $p < 0.1$), < 10% in the Sakishima region ($R^2 = 0.076$, $p < 0.05$), and

> 30% in the entire archipelago ($R^2 = 0.341$, $p < 0.01$), which indicates isolation-by-distance over the archipelago, but not at a given island. We also found that the westernmost site in the archipelago, IRMg, revealed linearized $F_{ST}P$ values ($LinF_{ST}P$), which are consistently above average regardless of Euclidean distance between IRMg and other sites (Figure 4). However, IRMg was not the cause of the lower fit, according to the sensitivity analysis.

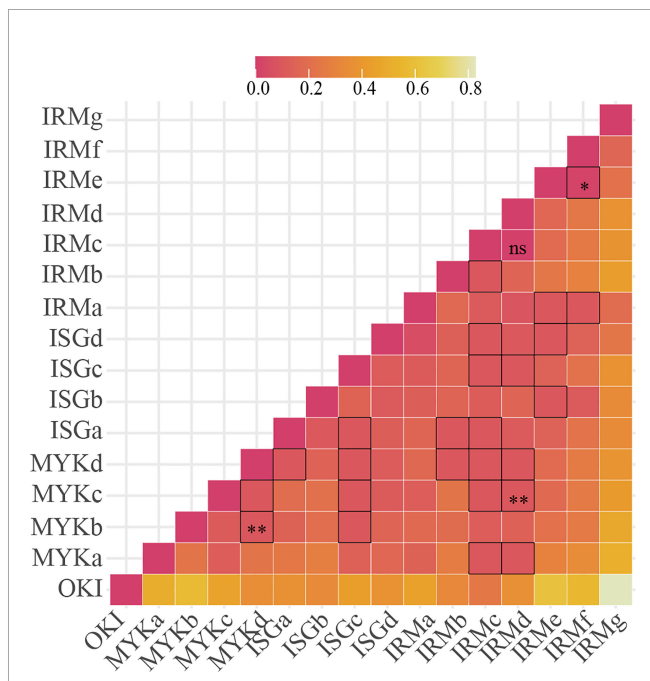


FIGURE 2 | Pairwise genetic differentiation values ($F_{ST}P$) revealed that strong connectivity exists among islands < 200km apart in the Ryukyu Archipelago. Of 25 site pairs with $F_{ST}P < 0.1$ (bold grids), 8 pairs exhibited intra-island connectivity, whereas OKI and IRMg are almost completely isolated. X- and Y-axes show site names, and the figure shows no directionality. P-values < 0.01 for all pairs, except for grids with *, **, and NS indicating $p > 0.1$, $0.05 < p < 0.1$, and not-significant, respectively.

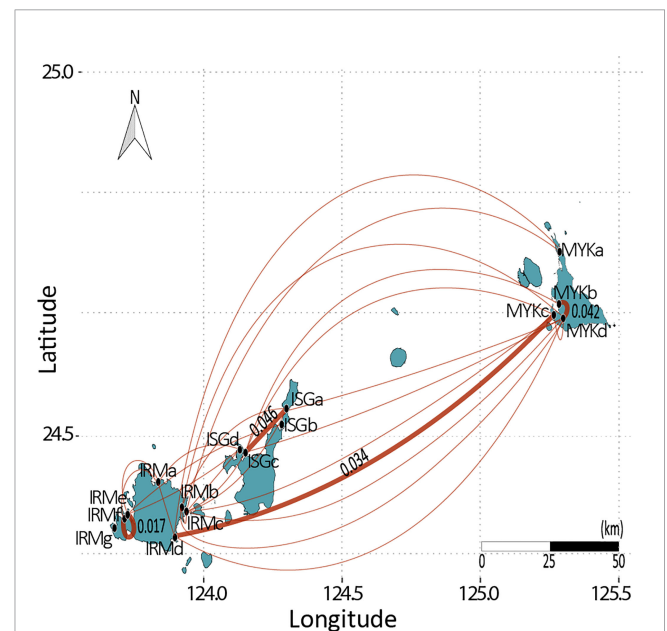


FIGURE 3 | Population pairs with $F_{ST}P < 0.1$ are projected over the map, and bold lines indicate pairs with $F_{ST}P < 0.05$, a threshold value indicating significant connectivity.

TABLE 2 | AMOVA summary shows that genetic variation is highest among individuals within populations, and that fixation indices are all significant with a tendency toward genetic isolation.

Source of variation	df	Sum of square	Variance Components	% of variation	Fixation index	P-value
Among populations	15	256.3	0.33	20.6	Fst	0.206
Among individuals within populations	338	838.8	1.20	74.7	Fis	0.940
Within individuals	354	27.1	0.80	4.8	Fit	0.974
Total	707	1122.2	1.61			0.0001

Structure Analysis

Bayesian analyses suggested 3 genetically homogeneous groups of individuals (genetic clusters) in the Ryukyu Archipelago (**Figure 5**): western Iriomote Island (WI), North Miyako Island (NM), and Okinawa Island (OKN). The majority of individuals (> 75%) on the west coast of Iriomote Island (WCI) had > 90% inferred ancestry from a local genetic cluster (WI), particularly at the westernmost site, IRMg, (> 95%). Site IRMf in the WCI area showed one individual with > 90% inferred ancestry from OKN. Most individuals (> 92%) at site OKI had > 90% inferred ancestry from a local cluster (OKN). OKI and IRMg exhibited homogeneous population genetic structures, highlighting the lack of common inferred ancestry between them. On the other hand, the remaining sampling sites showed heterogeneous genetic structure within each site. Miyako, Ishigaki, and the east coast of Iriomote Island (ECI) (**Figure 5**) exhibited individuals with > 80% of inferred ancestry from NM admixed with individuals having inferred ancestry from OKN and WI. For example, 27 individuals from the northern site of Iriomote Island (IRMa) included 2 individuals with inferred ancestry (> 80% here after) from OKN, 4 individuals with inferred ancestry from NM, 9 individuals with inferred ancestry from WI, and the remaining 12 individuals with mixed ancestries. We also found that very few inferred ancestries were shared within an island, particularly between WCI and ECI.

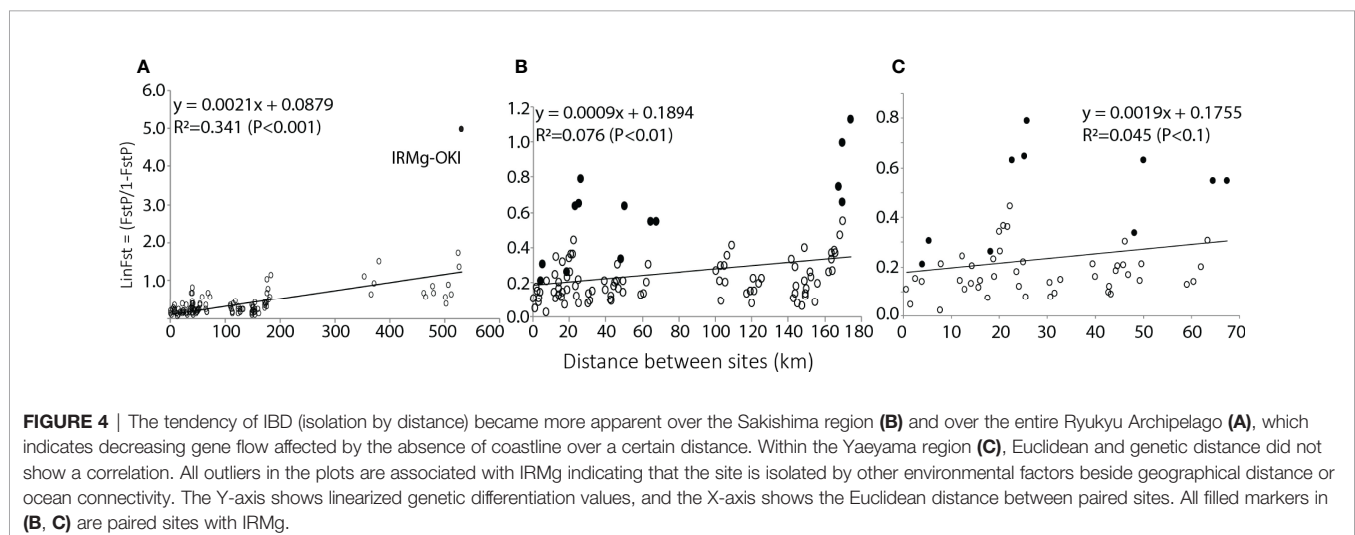
Contemporary Migration Rates

Contemporary migration rates (m) represent the frequency of genetic immigration in the last few generations between all pairs

of sites in the archipelago, which ranged from $m = 0.008$ to $m = 0.154$ ($m = 0.01$ implies that on average 1 individual among a sample of 100 per generation would be a migrant). The highest migration rate ($m = 0.154$) was found from Ishigaki Island (ISGa) to east of Iriomote Island (IRMb), followed by pairs among neighboring sites along the west coast of Iriomote Island (i.e., $m=0.149$ from IRMg to IRMf, and $m=0.114$ from IRMg to IRMe). However, migration rates in the opposite direction were substantially lower (**Figure 6**). The analysis also identified ISGa as the site with the highest frequency of emigrants to other sites ($\sum m_{ISGa(\text{Source})} = 0.489$). Furthermore, the analysis identified the potential for long distance (> 200km) propagule immigration from Okinawa Island (OKI) to Iriomote Island (IRMc) with relatively higher migration rates ($m = 0.052$) (**Figure 7**). The majority of migration rates were too low ($m < 0.05$) (Meirmans, 2014) to confirm genetic exchange between sites.

In Situ Drifter Experiments

Of 31 GPS drifting buoys released from a location just off the west coast of Iriomote Island, 6 drifters beached on the same island after an average of 23 days with high retention rates around the release points for the first 20 days (**Figure 8**), while one drifter took > 130 days to beach on the natal island coastline (**Figure 8, Table 3**). We confirmed 4 island-to-island dispersal beaching events, including one each on the coasts of Ishigaki Island (58 km in 12 days), Kuroshima Island (35 km in 9 days), Miyako Island (183 km in 58 days), and Yakabi Island (414 km in 109 days). We found that northeastward was the dominant direction of drifter movements north of the Yaeyama region



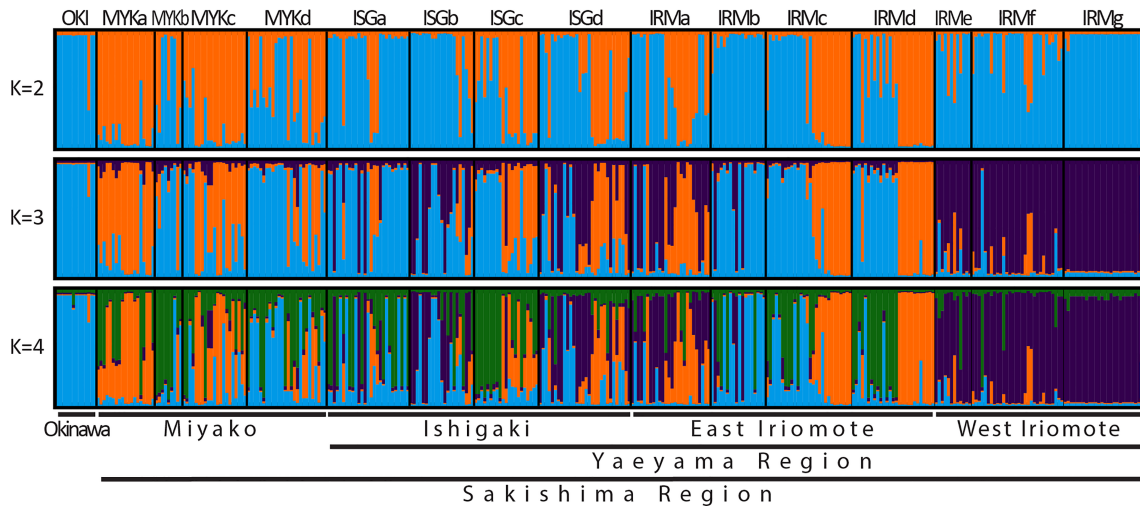


FIGURE 5 | The optimal number of genetic clusters was 3 ($K=3$); clustered with inferred ancestry from the west coast of Iriomote Island (WCI) (purple), from Miyako Island (orange), from Okinawa mainland (blue). Each bar represents an individual sample. The cluster from OKI was distributed throughout the Archipelago, but its ratio drastically declined at WCI (IRMe-g), while the cluster from WCI revealed very limited distribution, even among neighboring sites on Iriomote Island. Heterogeneous genetic structures were found at most sampling sites, except for IRMg and OKI, and such patterns were consistent from the results with $K=4$, but not with $K=2$ where both clusters are distributed throughout the archipelago.

(**Figure 9**). We also found that 60% of drifters were taken by the strong western boundary current, the Kuroshio Current, of

which 76% were carried out into the mid Pacific Ocean and were eventually lost due to battery failure (**Figure 10**). We also observed sporadic counter-current drifting southwestward with various trajectories. Frequent local eddies were also observed in the archipelago, causing dispersal periods unrelated to Euclidean distance from the deployment site. For example, we identified a drifter traveling > 1,000 km in 50 days while another traveled 183 km in 59 days.

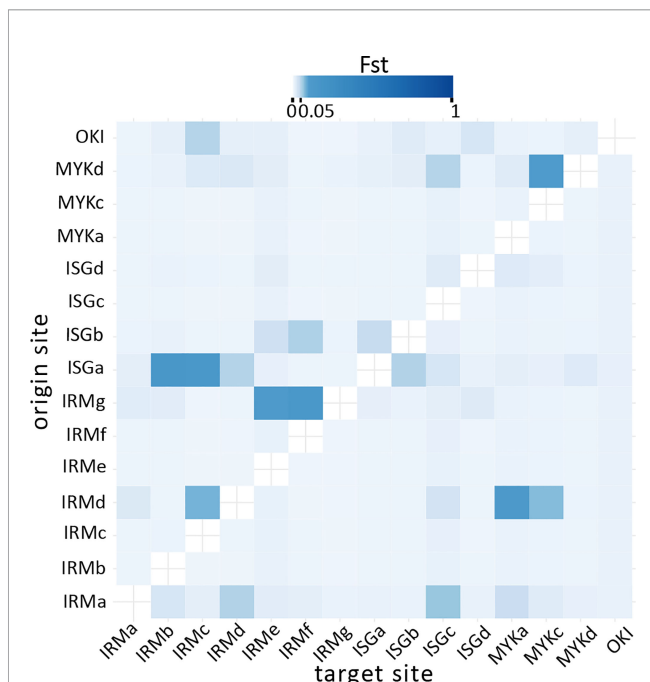
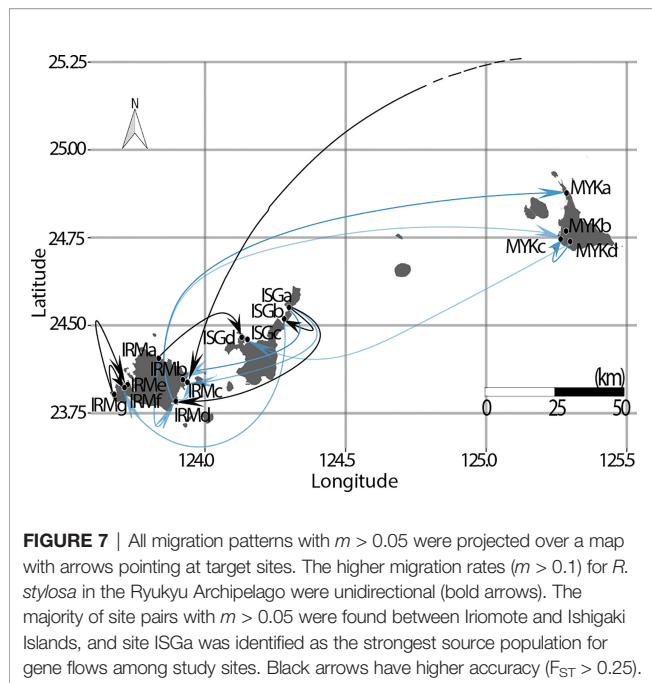


FIGURE 6 | Bayesian assignment test identified contemporary migration patterns in the Ryukyu Archipelago with posterior mean migration rates ($m = 0.01$ implying that 1 individual among a sample of 100 per generation is a migrant). The majority of site pairs showed lower m values (x-axis: target sites, y-axis: origin sites), and a few with higher, sporadic migration rates.

DISCUSSION

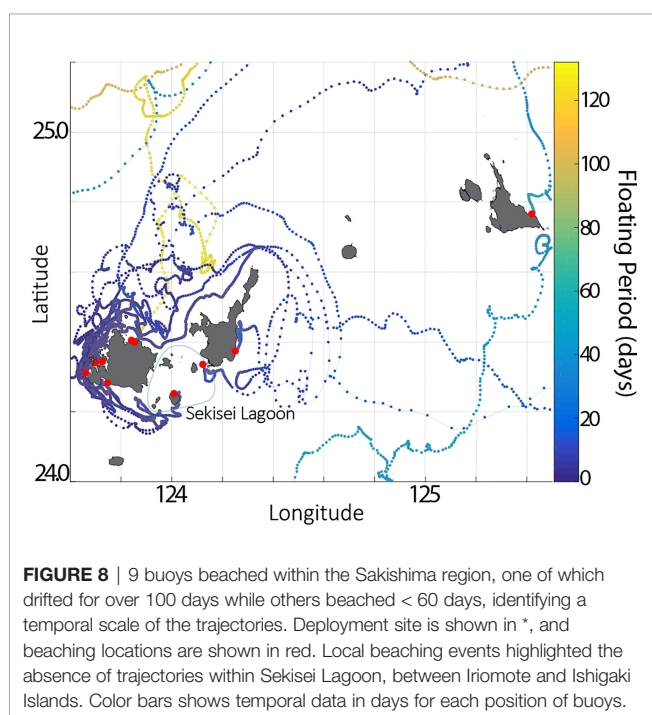
Genetic Diversity in Island Systems

Significant genetic disequilibrium found in this study may be due to high monomorphism caused by high rate of inbreeding expected in naturally fragmented population (Provan et al., 2008) or/and by selfing due to pollinator limitation (Bawa, 1990) both of which are common to *Rhizophora* species. The genetic variance (N_a), inbreeding coefficient (F_{IS}) and limited area of fringing mangroves in the current study all exhibited characteristics of habitats at the species range limit. The Ryukyu Archipelago is located at the northern edge of mangrove global distribution, and site OKI, showing the lowest genetic variance in the study ($A_r=1.2$), is the northernmost habitat of *R. stylosa*. Compared to a genetic connectivity study of *R. stylosa* in South China (Geng et al., 2021), the reduction of genetic diversity in this study area was apparent. Hence, *R. stylosa* in the Ryukyu Archipelago maybe subject to genetic drift and environmental selection due to population sizes limited by extreme and/or variable environments (Brown et al., 1996) at the edge of the species distribution. Based on genetic structures indicating



infrequent gene flow among sites (Figure 5), we suggest that the genetic variation at the time of establishment in the archipelago may already have been limited.

Lack of riverine inputs may also have effects on low genetic variation. The current samples were collected at fringing mangrove habitats in proximity to the open ocean, where there were no inputs from rivers. On the other hand, samples used by Islam et al. (2014) were collected from river basins located



further inland than our sampling sites. Over all, F_{IS} of this study was substantially higher and H_o was also significantly lower than values reported by Islam et al. (2014) (Table 1). Proximity to open ocean has been suggested to influence inbreeding and genetic diversity of mangrove populations, in that the most seaward habitats should have a higher likelihood of propagule dispersal and should exhibit higher genetic diversity (Triest et al., 2021a). However, genetic comparisons with those of Islam et al. (2014) revealed the opposite, and suggested very low likelihood of propagule dispersal among fringing mangroves in the island system via ocean currents, despite being directly exposed to ocean hydrodynamics. In general, riverine systems provide more suitable habitats for mangroves because the constant sediment supply from upstream expands available habitat areas for new propagules (Kadoya and Inoue, 2015). This, in turn, supports larger population sizes resulting in greater genetic variation within habitats (Kadoya and Inoue, 2015). Additionally, gene flow in riverine systems may be bidirectional because of interacting river flow with tidal fluxes, coastal currents, and wind, which supports genetic diversity within fluvial systems (Ngeve et al., 2017). On the other hand, fringing habitats lack terrestrial input and fluvial interactions with rivers. Thus, habitat areas are often limited, and demographic connectivity must rely predominantly on ocean currents, which limit genetic diversity due to a lack of connectivity among populations on island systems. Over all, genetic heterozygosity ($H_o > H_e$) and inbreeding coefficients (F_{IS}) in the current study indicated inbreeding/selfing as the dominant method to sustain population sizes, which suggests that propagule dispersal in the archipelago is restricted to local scale. Genetic variation revealed that fringing habitats of the Ryukyu Archipelago are naturally fragmented with higher potential for genetic isolation within the island system, making them vulnerable to environmental changes.

Sporadic Propagule Transport Throughout the Ryukyu Archipelago

Considering ecological characteristics of propagules that contribute to LDD, in addition to direct ocean access at each sampling site, we hypothesized a uniform level of genetic connectivity throughout the archipelago. However, contrary to our hypothesis, we found genetic structures indicating limited connectivity. The majority of sites in the Sakishima region (Iriomote, Ishigaki and Miyako Islands) displayed heterogeneous genetic structures in each population with inferred ancestries from all three bioregions (OKN, NM, WI) (Figure 5). Since the wind-pollination success rate of *R. stylosa* is low, structural heterogeneity may indicate that propagule immigration from other sites is consistently sporadic over many generations, and that the frequency of inbreeding/selfing is too high to achieve homogeneity. On the other hand, at the edges of the archipelago, both OKI and IRMg exhibited genetic structure with one dominant inferred ancestry (OKN and WI, respectively) indicating their genetic isolation. Overall, genetic structures and F_{IS} showed that inbreeding/selfing has consistently been the major reproductive method and population connectivity even within islands is extremely limited.

TABLE 3 | *In situ* drifter experiments showed about 30% of local beaching (Bold letters) with a wide range of temporal variation from 0 to 132 days.

ID	Deployment Time	Tide at deployment Incoming = 1 Outgoing = 0	Closest Distance to Shore (km)	Beched Coordinates (Lat, Lon)	Euclidian (km) between the beaching and deployment sites	Dispersal Duration	Notes
IRM-353-1	2018/08/07 9:08	0	0.5	–	–	–	
IRM-342-1	2018/08/08 7:10	0	0	24.3102, 123.6627	4.8	0.82	
IRM-343-1	2018/08/09 11:40	0	0	24.3422, 123.7293	4.6	0.94	
IRM-344-1	2018/08/13 10:45	0	0.02	–	–	–	
IRM-343-2	2018/08/14 11:05	0	–	–	–	–	
IRM-345-1	2018/08/14 11:05	0	0	24.3975, 123.8560	18.36	131.96	
IRM-346-1	2018/08/15 13:30	0	0.19	–	–	–	
IRM-347-1	2018/08/16 11:40	0	0.57	–	–	–	
IRM-348-1	2018/08/18 10:05	1	1.17	–	–	–	
IRM-349-1	2018/08/19 12:55	1	0.54	–	–	–	
IRM-350-1	2018/08/20 13:30	1	0	20.7030, 110.4308	1421	226.27	Guangdong, China
IRM-351-1	2018/08/21 10:45	1	0.92	–	–	–	
IRM-352-1	2018/08/22 10:35	1	0.81	–	–	–	
IRM-354-1	2018/08/25 14:00	1 spring tide	0	26.2179, 127.2397	413.8	108.7	Yakabi Island
IRM-355-1	2018/08/26 10:30	0 spring tide	0.57	–	–	–	
IRM-356-1	2018/08/27 13:40	0 spring tide	1.19	–	–	–	
IRM-357-1	2018/08/28 10:30	0	0.15	–	–	–	
IRM-358-1	2018/08/29 11:50	0	0.67	–	–	–	
IRM-359-1	2018/08/30 11:20	0	0	24.7645, 125.4220	182.8	58.79	Miyako
IRM-360-1	2018/08/31 07:00	1	0.37	–	–	–	
IRM-361-1	2018/09/01 17:21	1	0	24.3723, 124.2526	58	12.07	Ishigaki
IRM-362-1	2018/09/02 10:55	1	0	24.2813, 123.7498	9.8	5.45	
IRM-363-1	2018/09/03 17:20	0	1.13	–	–	–	

(Continued)

TABLE 3 | Continued

ID	Deployment Time	Tide at deployment 1 Outgoing = 0	Closest Distance to Shore (km)	Beached Coordinates (Lat, Lon)	Euclidian (km) between the beaching and deployment sites	Dispersal Duration	Notes
IRM-364-1	2018/09/04 7:30	0	0	24.2505, 124.0110	34.9	8.56	Kuroshima
IRM-365-1	2018/09/05 9:35	0	0.7	-	-	-	-
IRM-366-1	2018/09/06 15:55	1	0	33.2343, 126.3539	1018	49.89	Jeju, Korea
IRM-367-1	2018/09/07 10:20	0	0	24.3384, 123.7010	1.98	1.35	
IRM-368-1	2018/08/08 10:35	0	-	-	-	-	-
IRM-369-1	2018/09/09 10:50	0 spring	0	24.4031, 123.8435	17.8	5.47	
IRM-370-1	2018/09/11 11:00	0 spring	0.53	-	-	-	-
IRM-371-1	2018/09/12 10:40	0	1.12	-	-	-	-

Potential for LDD (> 200km) with successful beaching was observed, but they were all out of the species' maximum propagule floating period (100 days). Many un-beached buoys approached within < 1km of coastlines, but never beached, which indicated the influence of alongshore currents at coral reefs.

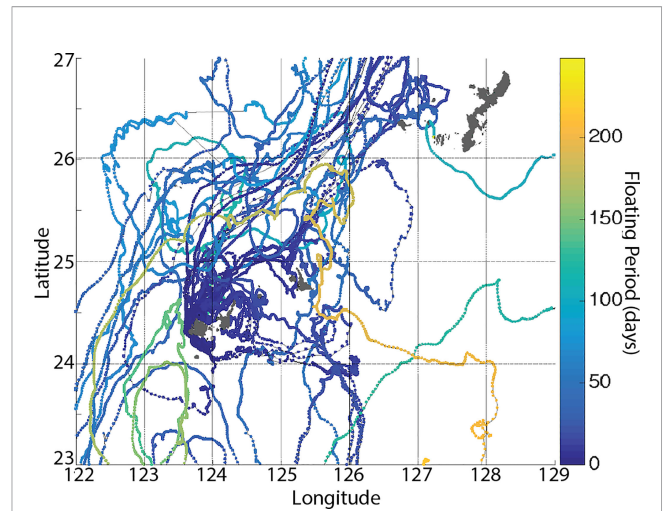


FIGURE 9 | The Ryukyu Archipelago, showing differences in concentration of trajectories within the island system, especially between west and east ends of the archipelago. Particles deployed from western Iriomote Island tend to stay on the west side of the archipelago, but showed frequent mixing within the Yaeyama region. The color bar shows temporal data in days for each position of the buoys.

Directionality and spatial range of historical genetic connectivity were interpreted based on shared inferred ancestries in populations. With > 80% of OKN inferred ancestry in an individual throughout the Ryukyu Archipelago, genetic exchange southwestward is suggested. On the other hand, inferred ancestry of WI is absent in Miyako and Okinawa islands,

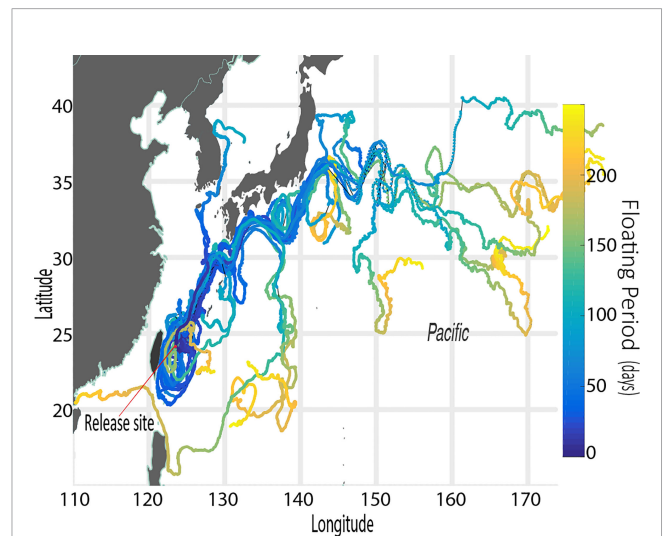


FIGURE 10 | Trajectories of 31 GPS drifting buoys over 250 days are shown. The retention period of buoys in the Ryukyu Archipelago is < 100 days on average and only a few stayed as long as 200 days. The Kuroshio Current is involved in trajectories of particles released from western Iriomote Island. Potential to travel as far as the mid Pacific Ocean was confirmed. The color bar shows temporal data in days for each position of buoys, and a red point shows the deployment location.

which suggests that northeastward genetic exchange declines drastically beyond Ishigaki Island (Figure 5). The decline of genetic exchange showed its correlation with distance between islands, according to the Mantel test between genetic differentiation and Euclidean distance (Figure 3). Hence, in the Ryukyu Archipelago, spatial range of > 100 km between islands in northeastward direction is identified as threshold for genetic discontinuity, and no population connectivity among islands was frequent enough to homogenize genetic structure within the archipelago. However, significantly high inbreeding coefficients from all study sites must be carefully considered since significant F_{IS} may cause additional populations to be inferred (Falush et al., 2003). Additionally, high F_{IS} and estimated selfing rates in the studied populations have been explained for *R. mucronata* (Triest et al., 2021b). The optimal K was 3 in this study, based on the Evanno method (Evanno et al., 2005), but according to the genetic structure with $K=2$, all islands are well admixed within the archipelago; however, not enough connectivity among islands was found to homogenize the genetic structure (Figure 5). On the other hand, at the edges of the archipelago, both OKI and IRMG exhibited genetic structure with one dominant inferred ancestry (OKN and WI, respectively) indicating their genetic isolation. Over all, genetic structures and F_{IS} showed that inbreeding/selfing has consistently been the major reproductive method and population connectivity even within islands is limited.

Reliable migrant ancestry assignment values ($m > 0.05$) of the last few generations between populations were found between 14 site pairs, mostly from Ishigaki to Iriomote Island representing an influx of immigrant alleles among populations via propagule dispersal. This southwestward pattern of propagule immigration from Ishigaki Island to ECI was also found in a locally abundant broadcast-spawning coral species, *Acropora tenuis* (Zayas et al., 2016). Low frequency of influx of alleles in recent generations was suggested over the archipelago, and inter-island influx was only found in a southwestward direction, as suggested from genetic structure. However, we must remain tentative with our results derived from only 7 loci, which is the lower limit of sample size suitable for BayesAss assignment (Wilson and Rannala, 2003). With a small number of loci (5 in Wilson and Rannala, 2003), accuracy of assignments is reasonable if migration is low ($m < 0.2$) and genetic differentiation is high ($F_{ST} > 0.25$) (Wilson and Rannala, 2003). Hence, migration ancestries with higher accuracy in this study were found only between 6 pairs (Figure 6). With obtained high F_{IS} values in the archipelago, this suggests that the influx of propagules from other populations is extremely low, but still contributes to maintain genetic diversity within populations in the archipelago.

Overall, in this study, contemporary gene flow patterns identified with higher migration rates ($m > 0.05$) seem to correspond well to the results of STRUCTURE, indicating that those sites with $m > 0.05$ have undergone consistent, sporadic gene flow over a historical timescale (Wilson and Rannala, 2003). Based on the “one-migrant-per-generation” rule (Mills and Allendorf, 1996), one immigrant per generation is enough to avoid harmful effects of local inbreeding in a population (Lowe

and Allendorf, 2010). Thus, populations with $m > 0.05$ and population size > 20 in the archipelago indicate that those populations may avoid harmful effects of local inbreeding/selfing, despite low genetic variation and limited genetic heterozygosity.

Spatiotemporal Interpretation of Propagule Immigration Based on Ocean Currents

Numerical ocean modeling and Lagrangian particle tracking methods have been widely accepted to estimate larvae dispersal of marine organisms. A previous mangrove study utilizing a numerical modeling method identified predominant coastal propagule transport along several continental coastlines, and island systems that are stepping stones to transpacific connectivity for mangrove species (Van der Stocken et al., 2019). However, the process of detachment from and settlement in nearshore areas before and after dispersal have not yet been examined. Despite the lower statistical power compared to numerical ocean modeling, the release-recapture method utilizing drifting buoys shows beaching events as part of settlement. *Rhizophora stylosa*'s propagules are about 23 cm in length, weigh about 35 grams, tend to maintain an efficient vertical floating orientation in higher salinity while fully submerged at the surface (Clarke et al., 2001). Hence, drifting buoys are suitable surrogates for mangrove propagules to simulate a subset of dispersal trajectories to estimate potential connectivity via propagule dispersal among islands, and to interpret genetic results at a current time scale.

Of 31 drifters deployed from just outside the reef at WCI, 4 trajectories revealed potential for inter-island dispersal with various destinations in the archipelago (Ishigaki, Kuroshima, Miyako, and Yakabi Islands), and 6 trajectories showed within-island dispersal (Table 3). Mangrove sites that are < 100 km apart were indicated as neighboring sites based on genetic analyses; thus, we suggest that beaching events on Ishigaki Island (~58 km in 12 days) and Kuroshima Island (~35 km in 9 days) represent the potential extent of local dispersal, and those on Miyako Island (~180 km in 59 days) and Yakabi Island (~400 km in 109 days) represent potential for LDD. Those potentials for dispersal are further supported by the estimated minimum and maximum floating periods of propagules considering that they play significant roles restricting dispersal range (Van der Stocken et al., 2019). Although there have been no studies to our knowledge on *R. stylosa*'s maximum floating and viability periods, we assume that *R. stylosa* shares propagule traits with *R. mucronata*, morphology of which is almost indistinguishable from that of *R. stylosa* (Spalding et al., 2010). Hence, the potential for northeastward LDD among islands is supported by *R. stylosa*'s maximum dispersal viability (150 days, median: 70 days), even though the survival rate of propagules is also suggested to decrease as the dispersal period increases (Drexler, 2001). However, no genetic result confirmed long-distance connectivity from WCI to other islands. Hence, northeastward LDD in the archipelago was not documented by

this study. On the other hand, the retention period of buoys in the Yaeyama region was below the maximum floating period (**Figure 10**), which may increase the potential for beaching at local scale and may explain the admixed genetic structures in this region (**Figure 5**).

The genetic isolation of OKI is probably influenced by the Kuroshio western boundary current. The Kuroshio current flows northeastward about 150–200 km west of the Ryukyu Archipelago, and current velocity and location are not only seasonal, but also highly variable year by year (Uchiyama et al., 2018; Takeda et al., 2021). The majority of observed trajectories beyond Ishigaki Island following the Kuroshio current confirmed a tendency for fast, long-distance (< 100 days) northeastward dispersal without reaching any coast in the archipelago (**Table 3**). The limited distribution of the WI cluster on Miyako and Okinawa Islands maybe due to the Kuroshio current, with a temporal limitation imposed by propagule viability. These limited trajectories were also found with numerical ocean modeling in a previous study on the Ryukyu Archipelago (Uchiyama et al., 2018).

Local beaching events within 6 days on Iriomote Island were confirmed, except for one drifter that took > 131 days to beach on the coast 18 km from its deployment site. Drifter experiments highlighted the wide range of temporal variation in dispersal affected by local eddies. We suggest that the higher migration rate in WCI (IRMg-IRMf, IRMg-IRMe) and homogeneous genetic structure in the area (**Figure 5**) may be explained by the higher retention rate around the deployment site along the reef of WCI during the first 20 days (**Figure 9**). Retention by currents in WCI and a high proportion of self-recruitment were also hypothesized in a previous ocean-modeling study for coral dispersal in the archipelago (Uchiyama et al., 2018). Moreover, the absence of a trajectory from WCI to ECI indicated a potential local oceanic barrier, which may explain the genetic discontinuities we found between those areas. Using a numerical model, Uchiyama et al. (2018) also indicated lower particle density reaching ECI from WCI, which suggests a consistent local oceanic pattern.

The difficulties of propagule dispersal among sites were further suggested by observed alongshore currents. More than half the drifters (19) did not beach during our experiments, and their closest approaches to coastlines while drifting ranged from 20 m to ~1 km (**Table 3**). Most drifters reached reef edges, and drifted with the alongshore current without entering the reefs. It is important to note that shallow reefs even interfere with genetic exchange between close sites for coral species such as *Pocillopora damicornis* (Miller and Ayre, 2004) despite their floating capacities and higher abundance of larvae compared to mangrove propagules. Hence, we suggest that tendency of localized dispersal of *R. stylosa* is mainly due to its low propagule fecundity, despite the long floating and viable periods of propagules. Stokes drift, contrary to alongshore currents, also affects onshore transport (Monismith et al., 2018). However, these drifters were designed to eliminate effects of waves (Stokes drift); thus, representation of propagule dispersal with drifting buoys may have underestimated the success rate of actual propagules. It is also worth noting that

the present *in-situ* results represent subset of entire dispersal trajectories that are specific to seasons and release locations.

In this study, with a single empirical dataset with genetic results, we discussed how ocean currents may affect fine-scale connectivity among islands. We interpreted genetic discontinuities within and among islands with current patterns, the distance between islands, and propagule characteristics such as fecundity and floating periods; however, further statistical approaches are needed for a more robust understanding of genetic discontinuities in the archipelago. Fine-scale numerical models may be suitable to estimate tidal amplitude affecting inundation of mangrove habitats and cross-shore currents at the reef edge to predict propagule dispersal from distant habitats. However, including wave-current interactions and obtaining the right combination of environmental parameters, such as topological factors as parameters for the model to achieve a finer-scale evaluation for near shore currents is difficult (Kamidaira et al., 2017; Uchiyama et al., 2018). Overall, in this study we showed that island-to-island propagule dispersal of *R. stylosa* in the Ryukyu Archipelago is possible even without stepping stones, although in reality, it is not frequent enough to unify population genetic structure. We also found that the archipelago is isolated from global distribution. Our findings are based on mangrove habitats with little or no human destruction, which should serve as a warning for habitats on island systems that are subject to ongoing threats from deforestation and environmental changes. It is important to identify the spatiotemporal scale of propagule movement specific to each island system to accurately understand demographic connectivity among populations.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repository and accession number(s) can be found below: <https://datadryad.org/stash>, doi:10.5061/dryad.bcc2fqzdh.

AUTHOR CONTRIBUTIONS

MT designed and conducted the majority of the project, including field sampling, main experiments, data analysis, and manuscript writing. YN provided assistance in genetic experiments and analysis and contributed to manuscript writing. SM contributed to the designing *in situ* experiments and provided guidance for manuscript writing. All authors contributed to the discussion and interpretation of data, and contributed to the article and approved the submitted version.

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