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Spatial genetic structuring and demographic history of the little spinefoot *Siganus spinus* in the Western Pacific

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Abstract: Studies have suggested that fishes with broad distribution ranges, long pelagic duration, and great mobility in their early life histories may have an advantage in their ability to return to their natal reef habitats. *Siganus spinus* is a siganid fish with a broad dispersal strategy, which may also exploit its great mobility to enhance self-recruitment. Variations in mitochondrial DNA sequences were analysed to estimate the genetic structure and demographic history of *Siganus spinus* in the West Pacific. In total, 446 nucleotide sequences, including the $tRNA^{Thr}$ gene, the $tRNA^{Pro}$ gene, and the first half of the control region, were analysed from 240 individuals sampled at five sites spanning the West Pacific Ocean; 37 haplotypes were identified. Our results showed significant geographic genetic structure as well as isolation by distance. Both neutrality tests and mismatch distribution indicated that the population expansion of *S. spinus* through the study area may have occurred in the late Quaternary. A dispersal strategy characterised by large juveniles and open-sea distribution may have contributed to a sudden population expansion after the last glacial period, along with an expansion of distribution.

Key words: demographic history, genetic structure, mitochondrial DNA, nucleotide sequence, *Siganus spinus*, Suku

INTRODUCTION

Pelagic larval duration (PLD) is the primary driver of dispersal among distant patches of habitat for many species of marine fish. The dispersal of individuals among populations is of great importance for population dynamics, population persistence, and species expansion (Treml *et al*. 2008). For effective management and conservation of marine resources, it is necessary to identify and quantify population structures and patterns of connectivity. Population genetics (i.e., the molecular approach) is the most powerful tool for estimating connectivity and population structure (Jones *et al*. 2009), as it is impossible to directly observe dispersal.

The little spinefoot, *Siganus spinu*s, is commonly found on coral reef flats throughout the Indo-Pacific region. It has the second broadest geographic distribution among siganids, after the forktail rabbitfish *Siganus argenteus* (Woodland 1990). Juveniles of several siganid species settle in shallow coastal waters during certain times of the year (Tsuda and Bryan 1973; Popper and Gundermann 1976; Kami and Ikehara 1979; Kanashiro *et al*. 1999). In the Okinawa Islands region, large numbers of *S. spinus* and *S. argenteus* juveniles, collectively called "Suku", recruit to a back-reef moat during a specific lunar phase in early summer. Large schools are caught at this time via purse seining. Most "Suku" are *S. spinus*, which demonstrates a strict seasonal and lunar-synchronised pattern of spawning behaviour (Tawada, 1988). Although the yield of "Suku" varies greatly among years and locations, a large harvest may be valued at 10 million Japanese yen (approx. US$100,000; Tawada 1988). A typical commercial "Suku" fishery can round up a school of juveniles by purse seining, catching hundreds of thousands of individuals in a single haul. These fisheries may be causing the population declines responsible for reduced yields in recent years. A decrease in spawner biomass due to overharvesting may have considerable effects on subsequent recruitment and the long-term sustainability of the fishery (Man *et al*. 1995). Iwamoto *et al*. (2009) showed no gene flow and not differentiation between Okinawa-jima and Ishigaki-jima localities by mitochondrial DNA control region sequence analysis. Priest *et al*. (2012) reported the Southern Mariana Islands population had self-recruitment. However, few studies have investigated the genetic structure of coastal fishes throughout the West Pacific region. To elucidate the genetic variability, population structure, and gene flow of wild siganid populations for the development of effective resource management measures. This study investi-

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gated the genetic variability, population structure, and recent evolutionary history of *S. spinus* in the West Pacific using mitochondrial DNA (mtDNA) control region sequence analysis. In this study, we discuss the influence of oceanic currents and PLD to population structure.

MATERIALS AND METHODS

Samples collection and DNA extraction

In total, 240 *Siganus spinus* individuals were collected between 2006 and 2010 at five sites spanning from the South Pacific to the Northwest Pacific, mainly along the Kuroshio Current and the North Equatorial Current (NEC) (Table 1; Fig. 1). Fish samples were chilled on ice, frozen, or preserved in 70–99% ethanol, and subsamples of approximately 50 mg minced muscle or fin were placed into individual 1.5 mL plastic test tubes containing 0.5 mL TNES-8M urea buffer (Asahida *et al*. 1996). Genomic DNA was extracted from the tissues using proteinase K digestion with phenol–chloroform and diethyl ether extraction (Imai *et al*. 2004).

Amplification and sequencing

The mtDNA control region was amplified via Polymerase Chain Reaction (PCR) using the primers L15926 (Kocher *et al*. 1989), L15923 (Shields and Kocher 1991), or L-*Siganus*-Thr (Iwamoto *et al*. 2015) and H16498 (Meyer *et al*. 1990) with BIO*TAQ*™ DNA Polymerase (Bioline Ltd.), *Ex Taq*™ DNA polymerase (TaKaRa Bio Inc.) or KAPA*Taq*™ EXtra DNA Polymerase (Kapa Biosystems). The following reagents were added to each PCR microtube: for BIO*TAQ*™, 1 µL template DNA, 12.5 pmol of each primer, 5 µL 10× NH_4 reaction buffer, 5 µL 10 mM deoxyribonucleotide triphosphate (dNTP) mixture, 4 µL 50 mM $MgCl_2$ solution, and 2.5 units of *Taq* polymerase; for *Ex Taq*™, 1 µL template DNA, 12.5 pmol of each primer, 5 µL 10× *Ex Taq*™ buffer, 5 µL 2.5 mM dNTP mixture, and 2.5 units of *Taq* polymerase; for KAPA*Taq*™, 1 µL template DNA, 12.5 pmol of each primer, 10 µL 5× KAPA*Taq* EXtra buffer, 1.5 µL 10 mM dNTP mixture, 3.5 µL 25 mM $MgCl_2$ solution, and 2.5 units of *Taq* polymerase. Each sample was brought to 50 µL with sterile distilled H_2O. PCR conditions consisted of initial plate heating (94°C, 2 min) followed by 30 cycles of denaturation (94°C, 30 s), annealing (48–56°C, 30 s), and extension (72°C, 1 min), then a final extension step (72°C, 7 min) in a thermal cycler (GeneAmp 9700; Applied Biosystems). PCR products were purified using ExoSAP-IT (USB Co.) or a PCR Product Pre-Sequencing Kit (USB Co.). Amplified DNA was sequenced on an ABI 3700 genetic analyser (Applied Biosystems) using BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems). All haplotype sequences were deposited in the DNA Data Bank of Japan (DDBJ).

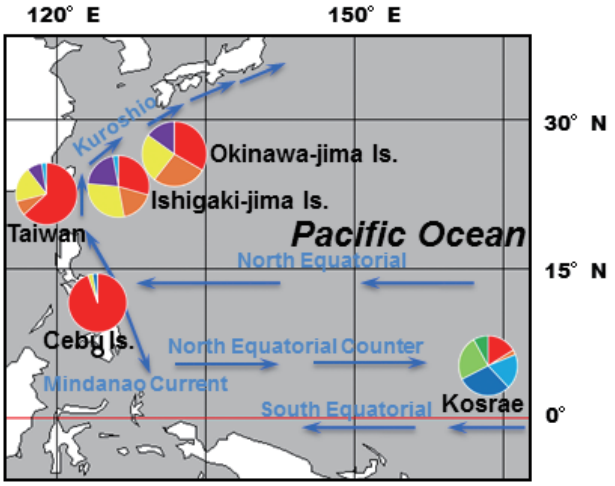

Fig. 1. Sampling sites, major haplotype frequencies (Ss1, 2, 3, 5, 22, 35 and 45) of represented pie-graph for *Siganus spinus* and sea currents. Numerals of two different circles were number of individuals.

Data analysis

Sequence data were aligned using ClustalW (Thompson *et al*. 1997) with the default parameters, and alignments were optimised manually. All types of substitutions were weighted equally in analyses. Population statistics were estimated using the program ARLEQUIN ver. 3.5 (Excoffier and Lischer 2010). The level of polymorphism for each population was estimated based on the number of substitutions, *h* (Nei 1987), π (Tajima 1983), and the mean number of nucleotide pairwise differences (*k*; Tajima 1983). To assess the relationship between haplotype and number of individuals, a network tree was created using Popart (Leigh *et al*. 2015) and drawn by hand.

Table 1. Summary of sampling localities, sample size (n), collection year and genetic diversity in five localities of *Siganus spinus*. *n*, sample size; N*h*, number of haplotypes; *h*, haplotype diversity; *k*, mean pairwise; SD, Standard deviation are in parentheses differences; π, nucleotide diversity

Locality	Abbribate	*n*	N_h	Geographic coordinate	Year	*h* (SD)	*k* (SD)	π (SD)
Okinawa-jima Island, Okinawa, Japan	OKI	55	17	26°30'N, 127°57'E	2006	0.902 (0.019)	2.793 (1.499)	0.006 (0.004)
Ishigaki-jima Island, Okinawa, Japan	ISG	52	17	24°23'N, 124°11'E	2006	0.888 (0.022)	2.673 (1.447)	0.006 (0.004)
Pingtung, Taiwan	TWN	48	14	22°02'N, 120°41'E	2007-2008	0.731 (0.064)	2.058 (1.174)	0.005 (0.003)
Cebu Island, Philippines	CEB	46	8	10°20'N, 123°51'E	2007	0.418 (0.090)	1.171 (0.768)	0.003 (0.002)
Kosrae Island, Micronesia	KOS	39	7	5°20'N, 163°01'E	2007, 2010	0.823 (0.028)	1.414 (0.884)	0.003 (0.002)
Total		240	37			0.863 (0.011)	1.995 (1.128)	0.004 (0.003)

Two approaches were used to infer demographic history. First, the null hypothesis of neutrality may be rejected when a population has experienced population expansion (Tajima 1989). Thus, two neutrality tests were applied to the mtDNA control region sequence data, Tajima's *D* test (Tajima 1989) and Fu's *Fs* test (Fu 1997). These tests and their significance levels were estimated using ARLEQUIN, based on 10,000 simulated resampling replicates. Alternatively, a population that has experienced a rapid expansion in the recent past exhibits a smooth, wavelike mismatch distribution with a star-like phylogeny (Slatkin and Hudson 1991; Rogers and Harpending 1992). We also used mismatch distribution analyses under the assumption of selective neutrality to evaluate possible growth and decline events in the historical population (Rogers and Harpending 1992; Rogers 1995). Past demographic parameters, including τ (time since expansion) and θ_0 and θ_1 (population size before and after expansion, respectively) (Rogers and Harpending 1992; Rogers 1995), were calculated using ARLEQUIN. In addition, Harpending's (1994) raggedness index was computed for each distribution, and its significance was tested using a parametric bootstrap approach with 10,000 replicates.

For distributions that did not differ significantly ($P > 0.05$) from the sudden expansion model, the demographic expansion parameter τ was a transformed estimate of actual time since population expansion, calculated using two formulae, $T = \tau/2u$ and $u = 2\mu k$, where μ is the mutation rate for the entire DNA region of interest, k is the length of the sequence (Rogers and Harpending 1992; Harpending 1994), and T is the time measured in years since expansion (Gaggiotti and Excoffier 2000). In the present study, mutation rates of 4–10% MY^{-1}, known as the molecular clock, were employed for the mtDNA control region of *S. spinus* (Iwamoto *et al*. 2012).

The population structure was examined by analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) using ARLEQUIN with a model of a single population group. Iwamoto *et al*. (2009) reported that high gene flow (pairwise F_{ST} 0.0036) detected between Okinawa-jima and Ishigakijima Islands (= southern Ryukyu). AMOVA and pairwise F_{ST} calculations combined these two localities in this study. AMOVA was also used to partition genetic variance within and between groups of samples. We estimated the proportions of variation among groups (F_{CT}), among populations within groups (F_{SC}), and within populations (F_{ST}). This hierarchical test can be used to reveal the degree of population subdivision (Schneider *et al*. 2000). Pairwise F_{ST} values based on uncorrected sequence differences (Wright 1951; Excoffier *et al*. 1992) were calculated to estimate the genetic divergence between paired populations, and the significance of F statistics for population comparisons was assessed with ARELEQUIN using 10,000 permutations. Significant levels were corrected by Holm method as the Bonferroni correction (Holm 1979). Gene flow among populations was estimated to be N_m, the number of migrants per generation between pairs of populations. N_m was calculated using the formula $N_m = (1–F_{ST})/2F_{ST}$ (Hudson *et al*. 1992). Pairwise values were transformed using Rousset's genetic distance equation $F_{ST}/(1–F_{ST})$ (Rousset 1997) and then plotted against the geographical distance between sampling sites to test isolation by distance (Wright 1943; Slatkin 1993). Under the stepping-stone hypothesis, genetic differentiation can build up between distant populations even while adjacent populations remain indistinguishable due to high pairwise gene flow. In such cases, a positive relationship may exist between geographic and genetic distance (Palumbi 2003). The slope,

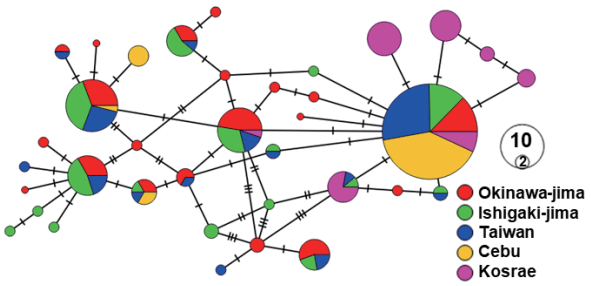

Fig. 2. Haplotype network presenting the median joining network with PopART among 37 haplotypes of *Siganus spinus*.

Table 2. Haplotype composition of five localities of *Siganus spinus*.

	Haplotype																		
Locality	Ss1	Ss2	Ss3	Ss4	Ss5	Ss6	Ss7	Ss8	Ss9	Ss10	Ss11	Ss12	Ss13	Ss14	Ss15	Ss16	Ss17	Ss18	Ss19
Okinawa-jima	11	9	8	5	5	3	2	2	2	1	1	1	1	1	1	1	1		
Ishigaki-jima	10	6	10	2	7	5	1											2	1
Taiwan	24	3	7	2	3	1	1		1						1				1
Cebu	35		1				2												
Kosrae	6	1																	

	Haplotype																		
Locality	Ss20	Ss21	Ss22	Ss23	Ss24	Ss25	Ss26	Ss27	Ss28	Ss29	Ss30	Ss31	Ss32	Ss33	Ss34	Ss35	Ss45	Ss48	Total
Okinawa-jima																			55
Ishigaki-jima	1	1	1	1	1	1	1	1											52
Taiwan			1	1					1	1									48
Cebu											4	1	1	1	1				46
Kosrae			7												11	9	3	2	39

intercept, and significance of this isolation relationship were assessed via reduced major axis regression and the Mantel test (10,000 permutations) using Isolation by Distance Web Service version 3.21 (www.bio.sdsu.edu/pub/andy/IBD.html; Jensen *et al*. 2005). Geographic distances were estimated to be the shortest distance in kilometres between the midpoints of two sampling sites using the path tool in Google Earth 5.2 (available at http://earth.google.com).

RESULTS

DNA variation and genetic variability

In total, 445–446 bp were sequenced from each of 240 *Siganus spinus* specimens collected at five sites. The obtained sequences included the $tRNA^{Thr}$ gene (44 bp), the entire $tRNA^{Pro}$ gene (69 bp), and the first half of the control region (332–333 bp). A consensus length of 446 bp was determined based on deletion. In total, 37 haplotypes were identified (DDBJ Accession Numbers AB713440 to AB713495). One haplotype (Ss1) was common to 86 individuals (35.83%) (Table 2; Fig. 1). The value of *h* ranged from 0.418 in the Cebu to 0.902 in the Okinawa-jima population. The level of π ranged from 0.002 in Cebu and Kosrae to 0.006 in the Okinawa-jima and Ishigaki-jima locations. Overall, the mean *h* and π among 240 individuals were estimated to be 0.863 and 0.004, respectively (Table 1). A haplotype network was constructed with the median joining network method, which indicated that these localities not fell in to the category of evolutionally significant unit (ESU) (Fig. 2).

Historical demography

Tajima's *D* and Fu's *F*s were determined. Neutrality indices calculated for each location and all samples are shown in Table 3. Significant negative values were observed in Okinawa-jima (*F*s = –6.267, *P* = 0.010), Ishigaki-jima (*F*s = –5.782, *P* = 0.011) and Taiwan (*F*s = –5.828, *P* = 0.009), as well as in the pooled sample (*D* = –1.622, *P* = 0.010 and *F*s = –2.671, *P* = 0.000).

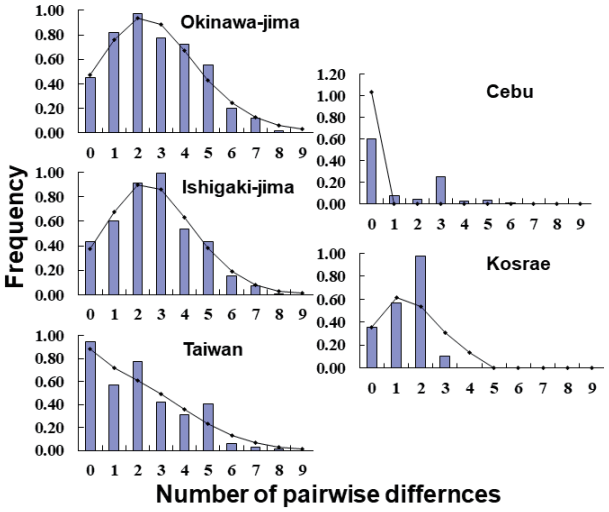

Fig. 3. Pairwise mismatch distributions for *Siganus spinus*. The observed frequencies (bar) and the expected mismatch distributions under a model of sudden expansion (solid line) are shown.

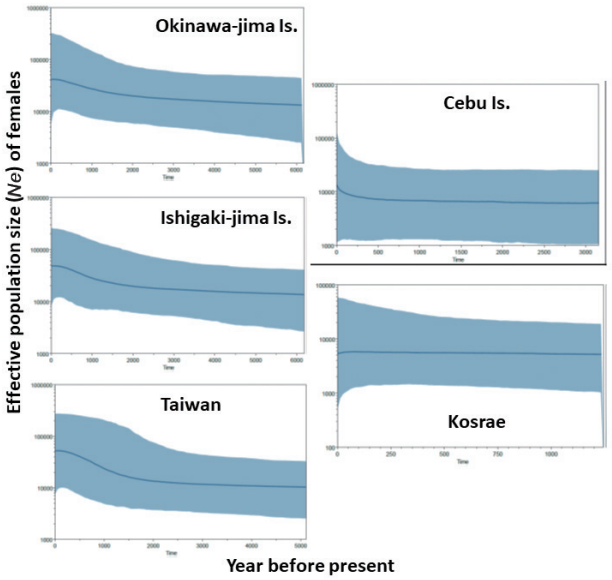

Fig. 4. Bayesian skyline plots analysis with implemented in BEAST on five localities of *Siganus spinus* based on mitochondrial control region sequences. The thick solid line depicts the median estimate and margins of the shape area represent the highest 95% posterior density intervals.

Table 3. Results of the mismatch analysis and neutrality tests for *Siganus spinus* localities.
τ, expansion parameter; obs. mean, mismatch observed mean; θ_0, θ_1, mutaion parameter before (θ_0) and after (θ_1) expansion; Ragged, raggedness index of Harpending (1994); Significant values ($P < 0.05$) are shown in bold. Corresponding P-values for each parameter are in parentheses

Locality	Demographic parameters				Test of goodness of fit	Neutrality tests	
	τ	obs.	θ_0	θ_1	Ragged (*P*)	Tajima's *D* (*P*)	Fu's *F*s (*P*)
Okinawa-jima	2.428	2.793	0.794	14.512	0.017 (0.780)	-0.447 (0.371)	**-6.267** (0.010)
Ishigaki-jima	3.033	2.673	0.004	16.035	0.025 (0.500)	-0.413 (0.391)	**-5.782** (0.011)
Taiwan	3.703	2.058	0.005	3.063	0.036 (0.830)	-1.063 (0.155)	**-5.828** (0.009)
Cebu	0	1.171	0.325	3414	0.347 (0.860)	-1.217 (0.091)	-2.501 (0.075)
Kosrae	1.75	1.414	0	6864	**0.247** (0.000)	0.501 (0.730)	-1.151 (0.259)
mean	1.844	1.995	0.226	2062.322	**0.051** (0.030)	**-1.622** (0.010)	**-2.671** (0.000)

Mismatch distributions were graphed for the entire sample and for each locality to determine whether the distribution conformed to a model of sudden expansion (Slatkin and Hudson 1991; Rogers and Harpending 1992). The mismatch distributions for most localities were unimodal, except for the Taiwan and Cebu localities, which appeared ragged (Fig. 3). The shape of the distribution suggested that a recent sudden population expansion may have occurred, and the parameters of the mismatch distribution implied all localities, excluding the Kosrae (Table 3). The τ values ranged from 0 to 3.703 (Table 3), signifying a population expansion approximately 7,400–42,500 years ago. The estimated effective female population size after expansion (θ_1) was markedly higher than before expansion (θ_0) for *S. spinus*, supporting a sudden population expansion. The Effective population size (*Ne*) of females were estimated with Bayesian skyline plots program (Fig. 4). Cebu and Kosrae extremely differ *Ne* process from passed to present.

Population structure

Significant genetic differences among localities were found in all combinations (F_{ST} = 0.036 ~ 0.270, P < 0.009) (Table 4). The estimated gene flow (N_m) between the Southern Ryukyu and Taiwan (N_m = 13.388) was higher than that among the all populations (N_m = 1.353-11.695) (Table 4). The results of AMOVA statistically supported this proposed locality grouping the Southern Ryukyu, Taiwan, Cebu and Kosrae (F_{CT} = 0.1496) (Table 5). Pairwise geographic distances plotted against the corresponding genetic distances among the five localities exhibited a positive correlation, suggesting isolation by distance (r = 0.622) (Fig. 5). The Mantel test confirmed the existence of a significant relationship between genetic distance $F_{ST}/(1-F_{ST})$ and geographic distance among these populations (P < 0.0001).

DISCUSSION

Our analyses of *Siganus spinu*s populations showed that the overall h values ranged from 0.731 to 0.902, excluding samples from the Cebu population. The Cebu population was an outlier, with a value of 0.418, comparable to reef and amphidromous fishes, e.g., the goldlined spinefoot *Siganus guttatus* (h = 0.955; Iwamoto *et al*. 2012), the mottled spinefoot *S. fuscescens* (h = 0.864; Iwamoto *et al*. 2015), the neon damselfish *Pomacentrus coelestis* (h = 0.956; Liu *et al*. 2008), grey demoiselle *Chrysiptera glauca* (h = 0.7540; Fauvelot *et al*. 2003), speckled butterflyfish *Chaetodon citrinellus* (h = 0.949; Fauvelot *et al*. 2003), and *Hypseleotris cyprinoides* (h = 0.993; Tomita *et al*. 2016). The π value (0.004) was similar to *C. glauca* (π = 0.0039; Fauvelot *et al*. 2003) but was an order of magnitude lower than *S. guttatus* (π = 0.020; Iwamoto *et al*. 2012), *S. fuscescens* (π = 0.013; Iwamoto *et al*. 2015), *P. coelestis* (π = 0.010; Liu *et al*. 2008), *C. citrinellus* (π = 0.0139; Fauvelot *et al*. 2003), and *H. cyprinoides* (π = 0.0136; Tomita *et al*. 2016). Imai and Numachi (2002) suggested h and π values as the genetic variability are related to population size. Grant and Bowen (1998) suggested population history scenarios based on h and π values. The high h and low π that characterise *S. spinus* attributed to recent population expansion after a period of low effective population size. Fauvelot

Table 4. Pairwise F_{ST} values (below the diagonal) and *Nm* values (above the diagonal) among four locations of Siganus spinus. Negative F_{ST} values were set to zero with Holm correction. Corresponding P-values for pairwise F_{ST} are in parentheses. Significance thresholds: *P < 0.05

Locality	Southern Ryukyu	Taiwan	Cebu	Kosrae
Southern Ryukyu		13.388	2.811	1.352
Taiwan	0.036 (0.000)		11.695	2.203
Cebu	0.151 (0.000)	0.041 (0.009)		2.739
Kosrae	0.270 (0.000)	0.185 (0.000)	0.155 (0.000)	

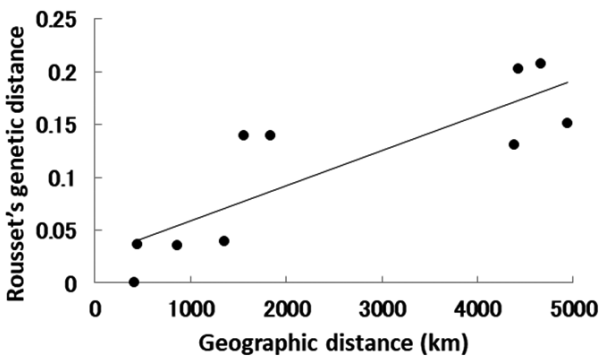

Fig. 5. Relationship between Rousset's genetic distance and geographic distances for the five localities.

Table 5. Summary of analysis of molecular variance (AMOVA) for mitochondrial DNA control region sequence of *Siganus spinus*. Abbreviations of localities are shown in Table 1. Significant values (P < 0.05) are shown in bold. df, dgrese of freedom; % var, percentage of variance. Groups defined according to the pairwise F_{ST} (Table 4).

Comparisons	Source of variance	*df*	% var	Fixation indices
All sites	Among localities	3	15.1	
	Within localities	236	86.48	F_{ST} = **0.1510**
(Southern Ryukyu), (TWN), (CEB), (KOS)	Among groups	3	14.96	F_{CT} = **0.1496**
	Among localities	1	0.17	F_{SC} = **0.0455**
	Within localities	235	84.87	F_{ST} = **0.1513**
(Southern Ryukyu, TWN), (CEB), (KOS)	Among groups	2	14.29	F_{CT} = **0.1430**
	Among localities	1	3.9	F_{SC} = **0.0455**
	Within localities	236	81.8	F_{ST} = **0.1820**
(Southern Ryukyu), (TWN, CEB), (KOS)	Among groups	2	13.99	F_{CT} = **0.1399**
	Among localities	1	2.41	F_{SC} = **0.0280**
	Within localititess	236	83.6	F_{ST} = **0.1640**
(Southern Ryukyu), (TWN), (CEB, KOS)	Among groups	2	6.88	F_{CT} = **0.0689**
	Among localiteies	1	8.73	F_{SC} = **0.0938**
	Within localities	236	84.38	F_{ST} = **0.1562**

et al. (2003) concluded that *C. glauca* has recently undergone rapid population growth after a strong bottleneck related to Holocene sea-level regression. The common haplotypes observed at most sampling sites suggest that a population bottleneck occurred, followed by a rapid expansion of populations from a modest number of founders (Avise 2000; Koike 2003). Pairwise sequence differences between *S. spinus* individuals showed a unimodal distribution with a range of 0–8 nucleotides, and the mismatch distribution did not deviate from the population expansion model in most populations. The two neutrality tests conducted on each population and the pooled sample largely yielded negative values, which are often the result of population expansion (Tajima 1989; Fu 1996). The combination of h and π, the star-like MST, and the good fit of the observed mismatch distribution to the sudden expansion model support the neutrality of *S. spinus*, favouring the demographic hypothesis and suggesting a recent population expansion. However, our results are unable to account for the low level of haplotypic diversity observed in the Cebu population (Table 1). This was probably due to a recent reduction in the size of this population (*cf*. Soliman *et al*. 2009), differences in demographic history caused by its geographic history, selection pressure among populations, or a combination of these factors. Genetic diversity may differ within and among species due to differences in their evolutionary histories (Fauvelot *et al*. 2003). In addition, although *S. spinus* may have experienced a sudden population expansion in the study area, our results were insufficient to explain the significant deviation from the model of expansion in the Kosrae (Table 3).

A rough estimation indicated that the population experienced extensive expansion 7,400–42,500 years before present. Paleoclimatic variation greatly altered the temperatures and levels of the oceans during the Last Glacial Maximum (LGM). When sea levels fell (120–140 m below the present level; Hewitt 2000; Lambeck *et al*. 2002), a strong decline in coral reefs inevitably occurred, accompanied by a drastic decline in reef fish populations due to the loss of their habitats. Various improvements in environmental conditions, such as the stabilisation of the water temperature, and sea levels, may have occurred after the end of the LGM. Thus, the population expansion of *S. spinus* could have occurred in the late Quaternary (i.e., the past one million years). Similar conclusions have been reported for the spotted sea bass *Lateolabrax maculatus* (Liu *et al*. 2006), neon damselfish *P. coelestis* (Liu *et al*. 2008), and Japanese Spanish mackerel *Scomberomorus niphonius* (Shui *et al*. 2009) in the West Pacific.

Marine organisms have long been thought to have no or low levels of genetic differentiation and structure over large geographic areas due to the PLD and the lack of obvious barriers in marine environments (Shaklee 1984; Palumbi 1992, 1994; Riginos and Victor 2001; Planes and Fauvelot 2002). As most adult marine fishes are relatively sedentary, dispersal between spatially fragmented habitats occurs primarily during the larval phase (Leis 1991; Bonhomme and Planes 2000). Siganid fishes exhibit low mobility and strict habitat preference (Tawada 1988); thus, dispersal must occur during their early life stages. *Siganus spinu*s has biological traits that favour long-distance dispersal, such as a PLD of approximately one month (Tawada 1988; Soliman *et al*. 2010), large juveniles (Kanashiro *et al*. 1999), and an open-sea dispersal strategy (Iwamoto *et al*. 2009). The dispersal of pelagic larvae over large geographic areas is usually explained as transport via ocean currents and the long PLD (Tomita *et al*. 2016). Strong ocean currents exist in our study area, namely, the NEC and the Kuroshio. Our results showed a population genetic structure in *S. spinus* distributed spatially across the West Pacific. The results of hierarchical AMOVA analysis indicated a high proportion of variance (20.61%) and highly significant population structuring. Johannes (1978) reported that most tropical marine fishes exhibit spawning behaviours, times and locations that favour the transport of their pelagic eggs and larvae offshore where predation is reduced, because intense predation puts heavy selective pressure on fishes in shallow coastal habitats. This ecological behaviour may have led to development of an oceanic dispersal strategy. This strategy, however, requires that the larvae return to shallow coastal waters. Accordingly, spawning is often concentrated at times of the year when prevailing winds or currents are at their weakest, thereby reducing the transport of larvae over long distances (Johannes 1978); i.e., the reproductive strategies of reef fishes depend upon a non-dispersal strategy characterised by self-recruitment (Jones *et al*. 1999). Several previous studies have indicated that reef fishes with high dispersal capacities (e.g., long PLD, large juveniles) exhibit high genetic divergence among populations in the absence of obvious barriers to dispersal (e.g., Bell *et al*. 1982; Planes *et al*. 1994; Shulman and Bermingham 1995; Riginos and Victor 2001; Fauvelot and Planes 2002; Planes and Fauvelot 2002). The biological traits of *S. spinus* favour long-distance dispersal as well as strong swimming ability to return to the natal reef. This strategy may not hinder larval dispersal even where strong ocean currents exist between spatially fragmented habitats.

The plot of genetic distance and geographic distance between sampling sites indicated isolation by distance. This suggested that *S. spinus* is at genetic equilibrium between dispersal and genetic drift (Slatkin 1993). Our results suggested that no gene flow among the Southern Ryukyu, Taiwan, Cebu and Kosrae. These observations demonstrated that a barrier exists preventing dispersal between the Southern Ryukyu and Taiwan. The Kuroshio forms a barrier isolating the islands of the

Ryukyu Archipelago from the main islands of Japan, Taiwan, and the Philippines, as marine organisms have difficulty crossing the strong boundary current between these regions (Aoki *et al*. 2008a; Aoki *et al*. 2008b; Imai and Aoki, 2012; Iwamoto *et al*. 2012, 2015; Imai *et al*. 2013; Kuriiwa *et al*. 2014; Tokuyama *et al*. 2020). This barrier may not prevent dispersal, as a reason for *S. spinus* juvenile size is bigger than reef fishes and form a school for dispersal phase and recruitment. In conclusion, our observations suggest that the genetic structure and connectivity of *S. spinus* populations are influenced by oceanographic phenomena as well as PLD and early life history. In addition, the management unit (MU) for resource management and conservation should utilize our results.

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