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ORIGINAL ARTICLE

Genetic connectivity of the coral-eating sea star *Acanthaster planci* during the severe outbreak of 2006–2009 in the Society Islands, French Polynesia

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Abstract

Occasional population outbreaks of the crown-of-thorns sea star, *Acanthaster planci*, are a major threat to coral reefs across the Indo-Pacific. The presumed association between the serial nature of these outbreaks and the long larval dispersal phase makes it important to estimate larval dispersal; many studies have examined the population genetic structure of *A. planci* for this purpose using different genetic markers. However, only a few have focused on reef-scale as well as archipelago-scale genetic structure and none has used a combination of different genetic markers with different effective population sizes. In our study, we used both mtDNA and microsatellite loci to examine *A. planci* population genetic structure at multiple spatial scales (from <2 km to almost 300 km) within and among four islands of the Society Archipelago, French Polynesia. Our analysis detected no significant genetic structure based on mtDNA (global $F_{ST} = -0.007$, $P = 0.997$) and low levels of genetic structure using microsatellite loci (global $F_{ST} = 0.006$, $P = 0.005$). We found no significant isolation by distance patterns within the study area for either genetic marker. The overall genetically homogenized pattern found in both the mitochondrial and nuclear loci of *A. planci* in the Society Archipelago underscores the significant role of larval dispersal that may cause secondary outbreaks, as well as possible recent colonization in this area.

Introduction

Acanthaster planci is notorious as a coral-eating sea star, which, although its population density is normally low, sometimes increases to >1500 ind. km^{-2} (15 ha^{-1}), a level generally defined as a population outbreak (Moran & De'ath 1992). If the rate of coral consumption by *A. planci* exceeds the rate of coral growth during such a population outbreak, the effect on coral reefs is devastating, with over 90% coral mortality on some Indo-Pacific reefs (Birkeland & Lucas 1990). Given the high fecundity of *A. planci*, at up to 70 million eggs per female per single spawning period, population outbreaks of adult *A. planci* could be caused by high survival rates during the early life stages (Birkeland & Lucas 1990). Its pelagic larval duration (PLD) is over 14 days long (Lucas 1973), followed by a juvenile stage of approximately 2 years before sizes are achieved (>15 cm diameter) that are easy to detect in the field (Lucas 1984). This long larval duration has been proposed as a reason for the observed successive population outbreaks. Once a population outbreak is initiated at a reef (termed the primary outbreak), the

primary outbreak population produces large numbers of larvae, possibly seeding downstream reefs and causing further population outbreaks a few years later (termed secondary outbreaks) (Kenchington 1977). This phenomenon is particularly well known in the Great Barrier Reef (GBR) in Australia (Birkeland & Lucas 1990) and the Ryukyu Islands in Japan (Yamaguchi 1987), where strong ocean currents, such as the East Australian Current, Kuroshio Current, and their associated currents, aid larval dispersal between distant reef habitats. To estimate larval dispersal in *A. planci*, several studies have examined the genetic structure of outbreak populations between different reefs using allozymes, mitochondrial DNA, or microsatellite markers. Indeed, previous population genetic and phylogeographic studies suggest there is a strong gene flow within both the GBR and Ryukyu Islands, whereas population differentiation and isolation-by-distance patterns are found among the different Pacific Islands (Nishida & Lucas 1988; Benzie & Wakeford 1997; Benzie 1999; Yasuda *et al.* 2009; Timmers *et al.* 2012; Vogler *et al.* 2013). Although a strong gene flow revealed by molecular markers alone cannot prove that the secondary outbreak hypothesis is true, genetic connectivity suggests important implications for larval dispersal. While a strong gene flow would imply a greater or lesser extent of larval dispersal, including possible secondary population outbreaks, significant population differentiation together with isolation-by-distance patterns would indicate larval dispersal limited by geographic distance, meaning the secondary outbreak hypothesis can be rejected.

From 2002 to early 2010, *Acanthaster planci* population outbreaks occurred progressively within the Society Archipelago in French Polynesia, forming the most intense and devastating outbreaks ever reported (Kayal *et al.* 2012). The minimum depth between the islands of French Polynesia is 1000 m (Duncan & McDougall 1976), making it physically impossible for adult *A. planci* to migrate between them: populations of *A. planci* can only be physically connected via larval dispersal. French Polynesia has a different geography and oceanography from intensively studied areas such as the Ryukyu Islands and the GBR. The Kuroshio current (up to 67 $cm \cdot s^{-1}$; Zhu *et al.* 2006) may enhance larval dispersal between different reefs of the Ryukyu Islands. Similarly the Eastern Australian Current flows may connect the continuous reefs of the GBR. On the other hand, around the Society Islands, the South Equatorial Current (SEC) is relatively weak (5–10 $cm \cdot s^{-1}$; Martinez *et al.* 2007) during the wet season spawning periods (Birkeland & Lucas 1990). Given these facts, *A. planci* population connectivity along the oceanic fragmented islands of the Society Archipelago, which has no strong western boundary current, is expected to be weaker than that in the Ryukyu Islands or the GBR, where secondary outbreaks are hypothesized. Previous studies have suggested that the Moorea population in French Polynesia is genetically isolated from other Pacific island populations such as the Fiji and GBR populations (Yasuda *et al.* 2009; Timmers *et al.* 2012; Vogler *et al.* 2013). Therefore, the successive *A. planci* population outbreaks observed in the Society Archipelago might either be secondary outbreaks or independent events caused by local factors (such as elevated chlorophyll concentrations; Houk *et al.* 2007).

The large amount of research on the gene flow of *Acanthaster planci* in the Indo-Pacific in order to estimate its larval dispersal makes it one of the most intensively studied coral reef invertebrates (Nash *et al.* 1988; Nishida & Lucas 1988; Benzie & Wakeford 1997; Benzie 1999; Vogler *et al.* 2008, 2012, 2013; Yasuda *et al.* 2009, 2011; Timmers *et al.* 2011). Most of these genetic studies use allozymes, microsatellites or mitochondrial DNA to reveal *A. planci* population genetic structure and gene flow over tens of thousands of kilometers. However, very few studies have examined multi-scale populations, including within-reef genetic structures of *A. planci*. Timmers *et al.* (2012) found an interesting 'reef-scale' population differentiation between forereef and lagoonal/backreef habitats in the Pearl and Hermas Atoll. This type of study underlines the importance of reef-scale sampling in genetic studies of *A. planci*, and the benefits of further research into possible ecological population differentiation within different reef systems.

In our study, we employed both the mitochondrial control region and microsatellite loci to examine the genetic structure of *Acanthaster planci* in French Polynesia. These *A. planci* genetic markers have never been used on exactly the same samples to examine their concordance directly. The two sets of markers yield different effective population sizes (nuclear microsatellite markers give a four times larger effective population size than mtDNA) and a different inheritance mode. Although mtDNA has a faster lineage sorting rate owing to its small effective population size and is normally the main indicator of population genetic structure (Zink & Barrowclough 2008), a combination of several microsatellite loci may have greater statistical power in the detection of genetic divergence (Larsson *et al.* 2009).

The aims of our study were first to examine population differentiation and isolation-by-distance patterns between *Acanthaster planci* populations at the reef- and archipelago-scale in the Society Archipelago in French Polynesia, and secondly to determine whether concordant patterns of genetic structure can be observed between mitochondrial DNA and microsatellite loci in *A. planci*. We hypothesized that if there is slight larval dispersal between islands, due to weak oceanic currents prevailing since colonization, genetic structuring and isolation by distance

could be detected at archipelago-scale around the Society Islands, opposite to what happens in the GBR and Ryukyu Islands. Moreover, if local selection or non-equilibrium existed during population outbreak, small genetic structuring could be detected at reef-scale.

Material and Methods

Sampling and DNA extraction

We sampled 240 individuals at nine stations located on four different islands (Tahiti, Moorea, Bora Bora and Taha'a) in the Society Islands, French Polynesia, at the time of the observed population outbreak in 2009 (Table 1). To integrate the regional and local scales, a hierarchical sampling design was established using samples collected from up to three sites on each of the four islands (three populations each from Tahiti and Moorea, two from Bora Bora and one from Taha'a), which were separated by distances ranging from as near as 1.6–270 km (Fig. 1). Samples were collected in June and July 2009, during the peak of the outbreak. Each station represented an area of approximately 1000 m^2 (10 × 100 m) located on the outer reef slope at a depth of between 5 and 15 m. Tube feet from each *A. planci* individual from each station were collected (16–30) and preserved in absolute ethanol. A solution of 10% Chelex resin was used to extract DNA from the ethanol-preserved sample solutions; the extracted DNA solution was further purified and then dissolved in TE (Tris-EDTA buffer) solution.

Mitochondrial analysis

The noncoding mitochondrial DNA (mtDNA) control region and partial 16s rRNA gene region were amplified with a polymerase chain reaction (PCR), using the COTS-ctrl-fwd 5′-CAAAAGCTGACGGGTAAGCAA-3′ primer and the COTS-ctrl-rvs 5′-TAAGGAAGTTTGCGA-CCTCGAT-3′ primer (Timmers *et al.* 2011). We performed PCRs using 10-μl final reaction volumes, containing 3 μl of dH_2O, 1 μl of undiluted template DNA, 0.07 μl of each primer (50 μM), and 5 μl of Kapa Taq Extra HotStart ReadyMix with dye (Nippon Genetics Co. Ltd, Koraku, Tokyo, Japan). Thermocycling was performed with an initial denaturation at 94 °C for 3 min, 35 cycles (94 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min), and a final extension for 5 min at 72 °C. PCR products were prepared for cycle sequencing using the Illustara Exostar (GE Healthcare, Tokyo, Japan), following the manufacturer's protocol. Sequencing reactions were conducted using COTS-ctrl-rvs and an alternate sequencing primer (Timmers *et al.* 2012) on an ABI 3730 capillary sequencer (Applied Biosystems Inc., Lincoln

Table 1. Summary information and statistics.

				mtDNA							Microsatellite				
Code	Island	Place name	Coordinate	N	H	h	He	π	Tajima's D	Fu' Fs	N	A	H_O	H_E	Fis
Ta	Tahiti	La Source	17°37′57.10″ S 149°37′19.36″ W	30	22	0.956	23	0.048	0.306	1.312	16	2.729	0.541	0.553	0.06
Tb	Tahiti	Vallee aux rougets	17°35′11.78″ S 149°37′44.52″ W	29	20	0.941	17	0.048	1.413	2.834	30	2.681	0.518	0.557	0.081
Tc	Tahiti	La Faille	17°37′5.70″ S 149°37′16.25″ W	26	19	0.967	30	0.046	0.744	1.147	30	2.810	0.551	0.573	0.049
MT	Moorea	Tiahura	17°28′58.32″ S 149°53′53.71″ W	6	6	1.000	–	0.059	1.022	−2.090	30	2.964	0.571	0.588	0.061
MH	Moorea	Haapiti	17°30′37.57″ S 149°55′35.19″ W	21	15	0.977	43	0.047	0.534	0.695	28	3.128	0.589	0.616	0.061
MV	Moorea	Vaipahu	17°28′47.17″ S 149°51′5.07″ W	30	24	0.977	43	0.053	1.087	−1.407	20	2.898	0.547	0.603	0.117
RM	Taha'a	Face Ceran	16°38′42.53″ S 151°26′33.70″ W	19	17	0.961	26	0.047	0.932	−0.559	30	2.732	0.551	0.586	0.084
Ba	Bora Bora	Hapiti NW	16°27′6.75″ S 151°46′43.35″ W	14	11	0.970	34	0.041	0.508	−0.075	29	2.728	0.572	0.588	0.048
Bb	Bora Bora	Tapu	16°30′8.12″ S 151°47′13.10″ W	29	17	0.942	17	0.042	0.193	−0.285	27	2.994	0.529	0.587	**0.119**

N is the number of samples, H is the mean number of haplotypes, h is haplotype diversity. He = 1/(1 − h) is the number of effective haplotypes, and π is nucleotide diversity. A is the number of effective alleles. H_O is observed heterozygosity and H_E is expected heterozygosity. Fis is inbreeding coefficients. Significant value ($P < 0.05$) are shown in bold.

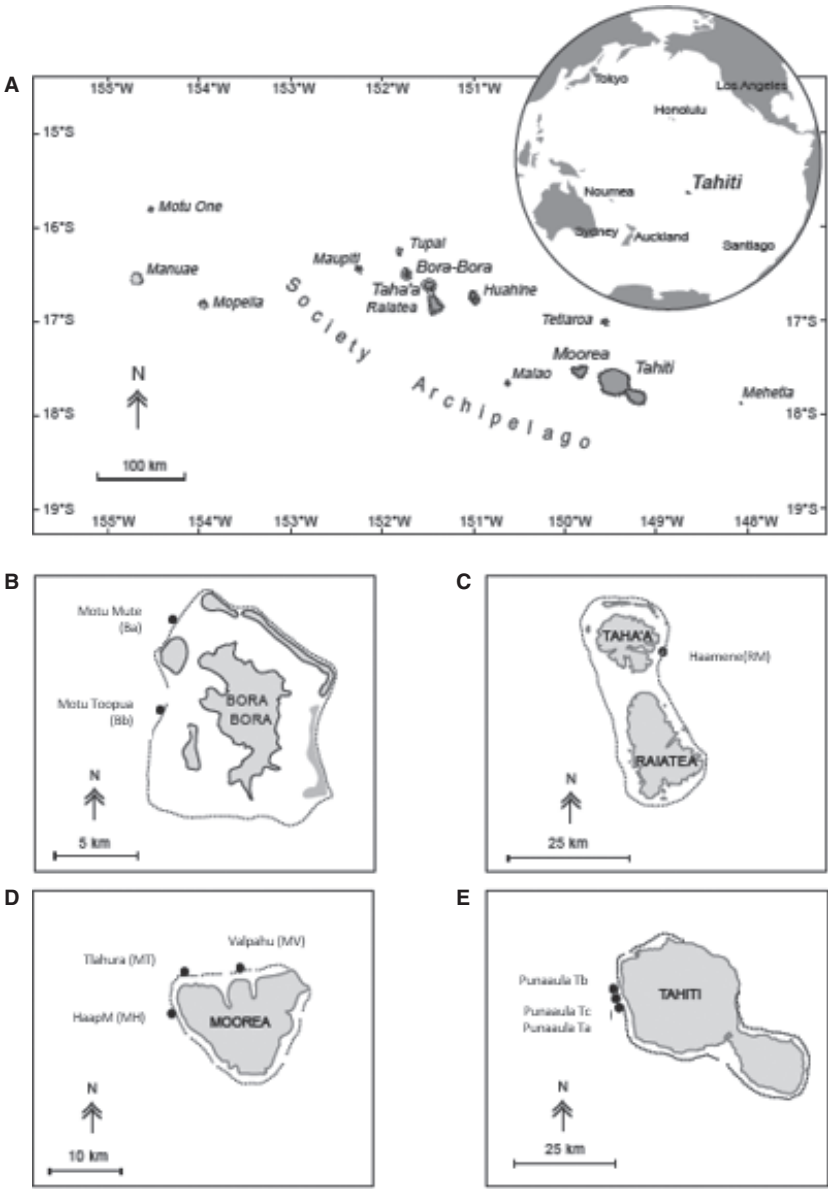

Fig. 1. Sampling sites used in this study.

Centre Drive, Foster City, CA, USA) using a BigDye Terminator v.3.1 Sequencing Kit (Life Technologies, Medical Center Drive, Rockville, MD, USA).

Standard population summary statistics were calculated using ARLEQUIN v3.5.1.3 (Excoffier & Lischer 2010). Measures calculated were the mean number of haplotypes, H; haplotype diversity, h; the number of effective haplotypes, He = 1/(1 − h); and the average number of differences between two random sequences from the same population, π. Tajima's *D* (Tajima 1989) and Fu's *Fs* (Fu 1997) were also calculated to compare *A. planci* control region diversity against neutral equilibrium expectations.

To visualize haplotype variability and clustering, median-joining haplotype networks, with a default weight of 10 applied to each character, were created using NETWORK v4.6.1.1 (Fluxus Technology Ltd).

Microsatellite analysis

We used six different Plexes for amplification of 23 microsatellites (Yasuda *et al.* 2006, 2007; Wainwright *et al.* 2012). 'Plex A' contained primer pairs for AP2457, AP5QS, AP654, AP32QS and AP5; 'Plex B' for AP30QS, AP6QS, AP19QS and AP22QS; 'Plex C' for AP12, AP9 and AP7; 'Plex D' for AP12QS, AP18QS, AP2592 and AP11QS; 'Plex E' for Etuko, Maki03 and Maki11; and 'Plex F' for Yukina05, Yukina06, Maki03 and AP1. Primers were fluorescently labeled with NED, VIC, PET or

FAM (Applied Biosystems). The loci were amplified using Type-It (Qiagen, Kachidoki, Tokyo, Japan) with denaturation at 95 °C for 5 min; 30 cycles of 30 s at 95 °C, 90 s at 50 °C, and 30 s at 72 °C; and a final extension for 30 min at 60 °C. Each 10-μl PCR reaction contained 5 μl of Type-It master mix and 0.1 μM of primers in deionized water. GENEMAPPER (Applied Biosystems) was used for genotyping all loci.

We calculated the number of effective numbers of alleles (A) and observed the heterozygosities (H_O) and expected heterozygosities (H_E) according to the Hardy–Weinberg equilibrium (HWE), using GENALEX ver. 6 (Peakall & Smouse 2006) (Table 1). The significance of any deviation from HWE was tested using FSTAT ver. 2.9.3.2 (Goudet 1995). Global tests for linkage disequilibrium were performed using a likelihood-ratio test with the level of significance determined by permutation (Markov chain parameters: 10,000 dememorization steps; 10,000 batches; 10,000 iterations per batch) using GENEPOP genetics software available on the internet (Raymond & Rousset 1995). Levels of statistical significance ($P < 0.05$) were adjusted according to a sequential Bonferroni correction for multiple comparisons (Rice 1989).

LOSITAN software (Antao *et al.* 2008), which performs an F_{ST}-outlier analysis to identify selected microsatellite loci, was used with 50,000 simulations to examine the neutrality of the markers. We estimated significance to a 95% confidence interval. Both the infinite alleles model (IAM) and stepwise mutation model (SMM) were used.

Genetic admixture analyses were conducted to examine possible genetic structures, using STRUCTURE 2.3.4 (Pritchard *et al.* 2010) based on 16 unlinked microsatellite loci. We used three different analytical models to examine possible genetic structure: (i) a; (ii) a model in which each collected location was a prior; and (iii) each island as a prior using the LOCPRIOR model. The LOCPRIOR model provides prior grouping information to enable us to observe genetic structure that is not always detectable using the normal admixture model (Hubisz *et al.* 2009); it does not tend to find structure when none is present and ignores sampling information when the ancestry of individuals is uncorrelated with prior information (Hubisz *et al.* 2009). For each analysis, $K = 1$ to $K = 4$ were run five times with different random seed numbers in order to examine the consistency of the results (1,000,000 replications and 500,000 burn-in).

Population differentiation analyses for genetic admixture model using a uniform prior both genetic markers

To examine population differentiation between population pairs, pairwise F_{ST} and Φ_{ST} were estimated using the mtDNA dataset and pairwise F_{ST} and D_{est} were estimated using the microsatellite dataset. All metrics except for D_{est} were calculated using ARLEQUIN ver. 3.5 based on 20,000 permutations. Φ_{ST} is an analogue of F_{ST}, taking genetic distances between haplotypes into account. The HKY+G+I nucleotide substitution model is not implemented in ARLEQUIN, and therefore the more inclusive Tamura–Nei model was used to calculate Φ_{ST}.

We also calculated pairwise D_{est} (Jost 2008) using SMOGD ver. 1.2.5 (Crawford 2010) with 1000 bootstrap replicates. Pairwise D_{est} values across the 16 loci used in this study were estimated by averaging their D_{est} values of 16 loci, to compensate for the possibility of different evolutionary rates. While F_{ST} and Φ_{ST} are fixation indices, D_{est} estimates actual genetic differentiation (Jost 2008).

We conducted three analyses of molecular variance (AMOVA) using traditional *F*-statistics to examine genetic structure along the Society Islands: (i) without a prior grouping; (ii) between different islands based on mtDNA F_{ST} and microsatellite F_{ST}; and (iii) for one of the Tahiti population (Tc) and the other populations, which was significantly differentiated based on pairwise F_{ST} analysis using microsatellites. Finally, we used a Mantel test (10,000 permutations) to determine whether genetic and straight-line geographic distances were correlated across the study populations using IBDWS ver 3.23 (Jensen *et al.* 2005). Pairwise F_{ST} values were used as mitochondrial genetic distance and D_{est} values were used as microsatellite genetic distance.

Results

Mitochondrial data

We obtained mtDNA haplotypes from a total of 204 *A. planci* individuals, each approximately 540 bp in length and containing a total of 115 substitution sites with 69 unique haplotypes. New sequences have been deposited in Genbank (Accession numbers. AB921571-AB921774). Overall gene diversity (Hd: 0.960) and haplotype diversity (π: 0.048) were high. None of the Tajima's *D* and Fu's *Fs* values was significant ($P < 0.05$) in the studied populations, suggesting neutrality and rejecting the hypothesis of recent expansion.

The haplotype network recovered four main distinct clusters, separated by at least 20 bp from each other, as well as some additional genetically distinct haplotypes (Fig. 2). However, there was no clear geographic affinity by haplotypic cluster, and many haplotypes were widely shared between the populations of the islands in the Society Islands. Neither global F_{ST} (-0.007, $P = 0.997$) nor Φ_{ST} (-0.008, $P = 0.724$) values were significant, indicating an absence of genetic structuring within the study area. The close values of F_{ST} and Φ_{ST} for mtDNA, despite

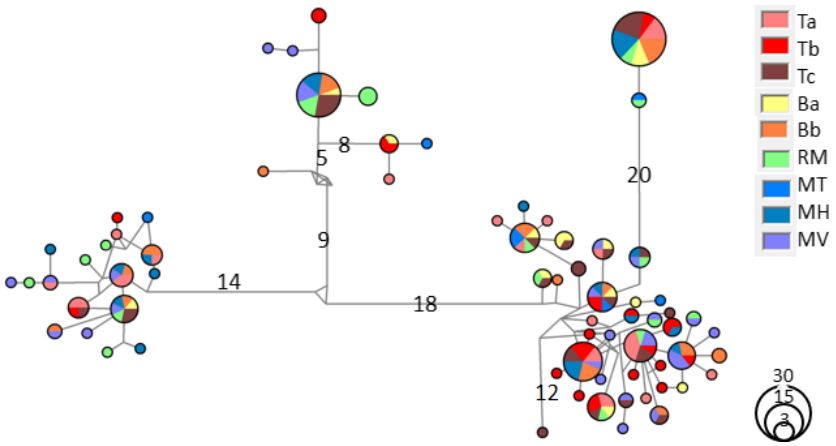

Fig. 2. Median-joining haplotype network based on the mtDNA control region. Numbers in the network indicate the number of mutations between haplotypes.

the existence of the four distinct genetic clusters, indicates that the migration rate is much greater than the mutation rate, given this spatial population partitioning. Likewise, none of the pairwise F_{ST} or Φ_{ST} values showed significant differentiation based on mitochondrial DNA.

Microsatellite data

Successful and stable PCR amplification and scoring was achieved for 18 microsatellite primer sets. However, we found significant linkage disequilibrium between a pair of loci (AP32QS and AP654). Two LOSITAN analyses indicated that Maki11 is a positive outlier (F_{ST} = 0.0144, P = 0.998 based on IAM; and F_{ST} = 0.0144, P = 0.999 based on SMM). Therefore, we used 16 neutral loci (Ap19QS, AP22QS, Ayu03, AP5, Ap5QS, AP654, AP30QS, AP6QS, AP11QS, AP18QS, AP2592, Etuko, Maki03, Y5, Y6, and Maki01) for the analysis. All populations except Bb were found to be in Hardy–Weinberg equilibrium (Table 1).

A small-scale but slightly significant genetic structure was found when using a global AMOVA (global F_{ST} = 0.006, P = 0.005) in the microsatellite analysis. Pairwise F_{ST} analysis showed that Tc is slightly differentiated (P < 0.05) from other populations (Table 2). Similarly, while the average pairwise D_{est} value across all samples

Table 2. Pairwise difference observerd with (a) mtDNA and (b) 16 microsatellite loci.

	Ba	Bb	MH	MT	MV	RM	Ta	Tb	Tc
(a) mtDNA Dloop pairwise F_{ST} (below) and Φ_{ST}(above)									
Ba		−0.036	−0.022	−0.021	−0.015	−0.016	−0.019	−0.013	−0.024
Bb	−0.016		−0.024	−0.020	−0.021	−0.023	−0.024	0.007	−0.001
MH	−0.010	−0.017		−0.027	−0.016	−0.009	−0.034	0.006	−0.014
MT	0.013	0.028	0.011		0.003	−0.068	−0.025	0.064	0.039
MV	−0.010	−0.006	−0.005	0.008		−0.011	−0.021	−0.006	0.009
RM	−0.012	0.007	0.006	0.004	−0.001		−0.013	0.039	0.039
Ta	−0.007	0.003	−0.004	0.022	−0.005	0.002		0.008	−0.009
Tb	−0.008	0.003	−0.010	0.005	−0.003	0.009	0.000		0.014
Tc	−0.025	−0.002	−0.004	0.027	−0.002	0.001	−0.010	0.007	
(b) Microsatellite pairwise F_{ST} (below) and D_{est} (above)									
Ba		0.012	0.008	0.004	0.005	0.013	0.007	0.003	**0.042**
Bb	0.010		0.011	0.008	0.000	0.011	0.000	0.003	0.014
MH	0.006	0.002		0.002	0.012	0.008	0.023	0.006	**0.043**
MT	0.000	0.003	0.001		0.000	0.008	0.015	0.004	**0.036**
MV	0.012	−0.001	0.005	0.004		0.006	0.013	0.006	**0.024**
RM	0.009	0.004	0.002	0.004	0.003		0.017	0.002	**0.024**
Ta	0.005	−0.002	0.006	0.006	0.008	0.007		0.010	0.018
Tb	0.004	−0.001	0.001	0.003	0.004	0.001	0.005		**0.040**
Tc	**0.028**	0.004	**0.012**	**0.017**	**0.016**	**0.008**	**0.012**	**0.015**	

P < 0.05 and D_{EST} > 0.02 are highlighted in bold. Pairs presented on a gray background when they remained significant after bonferonni correction for multiple test.

was 0.012, pairwise D_{est} values between Tc and the rest of the populations averaged 0.030, indicating relative population differentiation between Tc and other populations. This trend was found for nine of 16 microsatellite loci and, therefore, this differentiation was not caused by a specific outlier locus. Nonetheless, we detected no significant genetic structure between Tc and other populations in AMOVA (Table 3), nor did we find any evidence of genetic structuring among different islands based on AMOVA (Table 3).

Genetic admixture without a prior analysis showed well-mixed patterns for all K values (K = 2–4; Fig. 3.1). The value of mean probabilities was highest at K = 1 (LnP(D), −9530.5) and gradually decreased with increasing K, indicating panmixia. When the LOCPRIOR model was run using each population as a prior, LnP(D) peaked at K = 2 (LnP(D), −9523.4) and a high mean value of r > 3.5 was detected, indicating the sampled location does not provide significant information for determining population genetic structure (Fig. 3.2). Values of r near to 1 or <1 in the LOCPRIOR model indicate that prior groupings are informative, whereas large values of r indicate that either there is no population structure or that the structure is independent of the groupings (Hubisz *et al.* 2009; Pritchard *et al.* 2010). When different islands were set as a prior, LnP(D) was highest at K = 1 (LnP(D), −9530.1) and the LOCPRIOR model showed no difference between different islands, always having a high mean value of r > 6, indicating there is no genetic structuring among different islands (Fig. 3.3).

Neither mtDNA nor microsatellite data show evidence of isolation by distance (IBD) patterns (intercept = 0.013, slope = -1.054×10^{-4}, Z = −5.134, r = −0.120, P = 0.734 for mtDNA; and intercept = 0.027, slope = -1.082×10^{-4}, Z = 47.3109, r = −0.2515, P = 0.965 for microsatellite loci), suggesting there is no correlation between geographic distance and genetic distance for the studied populations.

Discussion

Contrary to our prediction that genetic differentiation and isolation-by-distance patterns would be found within the Society Archipelago, this study revealed an overall strong genetic connectivity for *Acanthaster planci* along the Society Archipelago on multiple scales, including reef-scale (<2 km) as well as archipelago-scale sampling. We found a good overall concordance between the different genetic markers. However, a slight genetic structuring was only found in the microsatellite loci, whereas an admixture of genetically distinct clusters was found in the mtDNA analysis.

Table 3. AMOVA results showing variance components (Var. comp), % variation (% Var.) and *F*-statistics for different grouping comparison.

		Among group				Among population within group				Within population			
Comparison	Genetic marker	Var. comp.	% var.	F_{CT}	P-value	Var. comp.	% var.	F_{SC}	P-value	Var. comp.	% var.	F_{ST}	P-value
Among different Islands	mtDNA	0.022	0.140	0.001	0.428	−0.136	−0.860	−0.009	0.680	15.91	100.7	−0.007	0.723
	microsatellite	−0.007	−0.170	−0.002	0.779	0.028	0.720	0.004	0.679	**3.95**	**99.5**	**0.006**	**0.005**
Tc *versus* others	mtDNA	0.040	−0.110	−0.110	0.670	−0.170	−0.170	−0.002	0.632	100.28	100.3	−0.003	0.068
	microsatellite	0.040	1.000	0.010	0.108	0.014	0.360	0.004	0.067	**3.95**	**98.6**	**0.014**	**0.005**

Comparisons in bold had P-values <0.05.

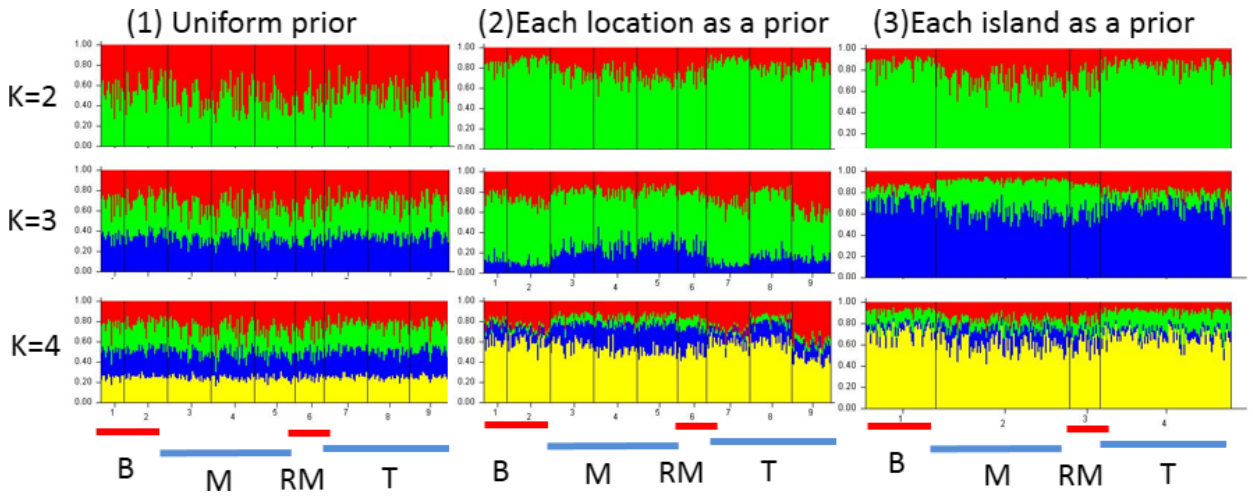

Fig. 3. Bar-plots showing results of the STRUCTURE analysis. K is the number of hypothetical clusters, each shown in a different color. (1) Genetic admixture analysis without prior information. (2) Each collection location as a prior using the LOCPRIOR model. (3) Each island as a prior using the LOCPRIOR model. Each individual is indicated on the X axis, and the different colors of the Y axis indicate the proportion allocated to each hypothetical genetic clade. B, Bora Bora (Ba, Bb); M, Moorea (MT, MH, MV); RM, Taha'a; T, Tahiti (Ta, Tb, Tc).

Overall strong genetic connectivity of populations along the Society Archipelago

We obtained concordant results from the mitochondrial D-loop region and 16 microsatellite markers, revealing a genetic similarity between *Acanthaster planci* populations of the Society Islands. An absence of IBD patterns in both genetic markers, as well as the high r value ($r > 4$) obtained using the LOCPRIOR model for locations or each island as a prior (Fig. 3), suggests the genetic connectivity is strong regardless of geographic location, whether within the same reef or separated by deep ocean within this region. The overall genetic homogeneity, with little or no genetic structure and without IBD, could be explained by high larval dispersal, which enhances gene flow between populations. This result suggests, even though there is no strong oceanic current, the long larval dispersal phase of *A. planci* (14 days or longer; (Yamaguchi 1973)) might mediate a large-scale dispersal between the studied populations. Larval dispersal of *A. planci* could potentially overwhelm the effect of genetic drift, resulting in the observed genetic homogeneity. This homogeneity could therefore be explained by outbreak populations originating from one or a few 'parental' populations and spreading via larval dispersal. However, as it is not possible to quantify accurately how far larvae travel per year based on this dataset, the data only partly support the secondary outbreak hypothesis.

Alternatively, it is also possible that *Acanthaster planci* has colonized the Society Islands very recently and populations are still in the early stages of differentiation. The retention of ancestral lineages within a species due to recent colonization of the Society Islands has been reported for the pearl oyster (Arnaud-Haond *et al.* 2003) and land snail (Goodacre & Wade 2001). A few other marine species with a pelagic larval phase, such as coral reef fish (Planes *et al.* 1993; Bernardi *et al.* 2001) and some coral species (Magalon *et al.* 2005), have also been found to have a similar genetic homogeneity within the Society Islands. The authors highlight that possible recent colonizations among the populations of the Society Islands may have resulted in genetically homogenized patterns. However, given that significant IBD patterns were detected for *A. planci* in previous studies conducted on wider scales, including the Society Islands (Yasuda *et al.* 2009; Timmers *et al.* 2012), and that the null hypothesis of recent expansion was rejected by a non-significant Tajima's *D* and Fu's *Fs* in the mtDNA analysis (Table 1), we considered it to be more likely that the effect of migration is larger than that of genetic drift in the Society Islands.

We concluded that the genetic similarity among the Society Island populations of *Acanthaster planci* examined in this study is primarily caused by larval dispersal, although it could be also caused by recent colonization. Whereas the applicability of the secondary outbreak hypothesis to the Society Islands cannot be verified by our data alone, it is notable that we observed the same patterns of genetic homogeneity as those for the Ryukyu Islands and GBR populations, where secondary outbreaks are suspected (Yamaguchi 1987; Yasuda *et al.* 2009).

Additional information obtained using different genetic markers

The same result, that overall genetic homogeneity along the Society Islands is probably due to high migration rate, was obtained using both mtDNA and microsatellite loci. However, some additional information can be obtained by using different genetic markers.

Distinct clusters found in the haplotype network of mtDNA (Fig. 2) suggested multiple origins for the *Acanthaster planci* populations of the Society Islands. We could not find any evidence of four distinct hypothetical ancestors in the microsatellite STRUCTURE analysis based on the genetic admixture model, which suggested that a single panmictic population is most likely (Fig. 3). This difference could have been the result of a historical weaker lineage sorting with the higher effective population size of nuDNA, together with a contemporary high migration rate masking any traces of ancestral lineages. Well-mixed patterns of distinct lineages (>20 substitutions) without geographic partitioning of mtDNA (Fig. 2) would indicate that the migration rate of *A. planci* is much higher than the mutation rate of the mtDNA control region. A previous study of the mtDNA region of *A. planci* sampled from the Hawaiian Archipelago and Johnston Atoll found no such distinct lineages, although lineages separated by five mutations were observed (Timmers *et al.* 2011). Reflecting these distinct lineages, overall nucleotide diversity was higher in the populations of the Society Islands ($\pi = 0.048$) than in the Hawaiian Archipelago plus Johnston Atoll populations ($\pi = 0.027$).

A small pairwise genetic differentiation between Tc and other populations was not observed in the mtDNA analysis but was detected using an analysis of 16 microsatellites. Pairwise D_{EST} values determined for Tc and other populations were higher than for pairs without Tc in more than half of microsatellite loci (nine of 16). Care needs to be taken in interpreting these data, as an AMOVA using a prior grouping for Tc and the other populations showed no genetic structuring with only 1% variation ($P = 0.108$) between groups (Table 3), indicating that the overall genetic differentiation is subtle. Nonetheless, it is notable that the Tc population is only separated from Ta by 1.59 km and from Tb by 3.62 km, suggesting that the genetic composition of *Acanthaster planci* populations could differ slightly over small geographic scales. One possible explanation is that selection is acting on the Tc population and preventing genetic homogeneity. However, we could find no obvious ecological difference, such as habitat variation or population density, between Tc and other populations. Further investigation into the various ecological and environmental parameters at each of the sampling sites, such as terrestrial stress and temperature, is required to determine whether selection is acting on the Tc population. However, given that an outlying locus showing possible selection was excluded from the analysis, the markers used are probably neutral and genetic differentiation is more likely to be caused by genetic drift. Variability in successful settlement caused by major fluctuations in population density might be a non-equilibrium process and result in the observed significant differentiation pattern. To confirm this hypothesis, further precise cohort analysis of populations collected from the same location, both before and during population outbreaks, would be useful in examining the effect of genetic drift associated with population expansion.

Finding a slightly higher F_{ST} value for the 16 nuclear loci than for mtDNA is itself an interesting phenomenon. In the ideal situation of strict uniparental organelle transmission, an even sex ratio, equal female and male migration rates, and an assumption of low mutation rates, the F_{ST} value of the mtDNA is expected to be higher than that of nuDNA, especially during population divergence, due to the much smaller effective population size of mtDNA (Larsson *et al.* 2009). F_{ST} values vary stochastically from 0.0 due to the sampling effects of division into subpopulations, and therefore a small sampling size (<50), together with stochasticity of the drift process, might result in the observed pattern. However, we cannot reject the possibility that the slightly higher F_{ST} value for nuDNA than for mtDNA is caused by nonequilibrium conditions or other factors, such as balancing selection on mtDNA and/or selection on microsatellite loci.

Conclusions

This study used both mtDNA and nuclear microsatellites to reveal a strong genetic connectivity of *Acanthaster planci* populations along the Society Archipelago. The fine-scale genetic structuring that was found only in the microsatellite analysis supports a need for further research into the possible effects of selection and genetic drift at times of major changes in the population density of *A. planci.*

The overall genetically homogenized pattern found along the Society Archipelago is similar to that observed in the Ryukyu Islands and GBR, where the secondary outbreak hypothesis is suspected to be relevant. Although further evidence is needed, the genetic data supports substantial larval dispersal among *Acanthaster planci* populations, highlighting the possibility of secondary population outbreaks within the Society Islands.

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