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Putative long distance gene flow of the Crown-of-Thorns Starfish in the Pacific Ocean

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Abstract

Population outbreaks of the corallivorous crown-of-thorns starfish (COTS), *Acanthaster 'planci'* L., are considered among the most important biological disturbances of tropical coral reefs. A local COTS outbreak, a "primary outbreak", can lead to so-called "secondary outbreaks" in adjacent coral reefs due to increased larval release and subsequent dispersion. Previous analyses have shown that in the Pacific Ocean, this dispersion may be geographically restricted to certain regions. Guam, an island in the western Pacific region, suffered from several severe COTS outbreaks in the last 50 years, and in this study we tested whether Guam is genetically connected with surrounding long distant regions. We used microsatellites to measure gene flow and genetic structure among 14 localities in the Pacific Ocean. Our results show substantial genetic structure between geographical regions. There was, however, a lack of significant genetic differentiation between localities separated by large geographic distances (e.g., Guam, Kingman Reef and Johnston Atoll) – a finding consistent with the existence of contemporary long distance larval dispersion and the gradual erasing of ancestral signatures of divergence. Our findings highlight the importance of addressing likely triggers of both primary and secondary outbreaks in conservation efforts using highly variable markers that provide enough variance to infer contemporary patterns of gene-flow and allow to implement programs that strive to control the growth and spread of *A. 'planci'* in the Pacific Ocean.

Introduction

The crown-of-thorns starfish (COTS), *Acanthaster 'planci'*, is a specialised coral predator and one of the most important biological threats to coral reefs throughout the Indo-Pacific (Pratchett et al. 2014). It has a complicated taxonomic history, initially considered a single widespread Indo-Pacific species (reviewed in Haszprunar & Spies 2014). Yet molecular data suggests that *Acanthaster 'planci'* is a species complex consisting at least four different species (Vogler et al. 2008), all of them showing internal phylogeographic structure, and one of which is largely restricted to the Pacific (Vogler et al. 2012; Vogler et al. 2013). Since formal description of these species is still pending, we here refer to the Pacific species as *Acanthaster 'planci'*.

COTS predatory behaviour has led to strong changes in the community structure of affected coral reefs (e.g., De'ath et al. 2012), promoting algal colonization, affecting fish population dynamics, and in numerous cases, causing the annihilation of entire populations of corals and the destruction of entire coral reefs (Kayal et al. 2012; Timmers et al. 2012). Massive outbreaks on the northwest coast of Guam in the late 1960s reduced coral cover down to 1% (Chesher 1969) and coral species richness decreased from 146 to 96 one year after the outbreaks (Randall 1973).

A. 'planci' populations undergo strong boom and bust cycles, characterised by pronounced oscillations in population density, with extended periods of low numbers of individuals per reef area, interspersed by short events of dramatically increased numbers of individuals in the same reef area, referred to as "outbreaks" (Brodie et al. 2005; Gérard et al. 2008; Kayal et al. 2012) when COTS predation causes negative trends in coral cover (reviewed by Pratchett et al. 2014). Although frequently studied, the origin, development and causes of COTS outbreaks remain largely unclear (Birkeland & Lucas 1990; Kayal et al. 2012; Pratchett et al. 2014; Vogler et al. 2013; Yasuda et al. 2009). Different authors (i.e., Benzie 1999a; Brodie et al. 2005; Gérard et al. 2008; Scheltema 1986) have highlighted the importance of larval survival and dispersion in explaining COTS outbreaks. A single female COTS can produce more than 60 million eggs per spawning season (Conand 1984) and this can result in more than 10 million fertilised eggs per year per mature female (Benzie et al. 1994). Therefore, a small increase in the survival rate of the COTS larvae could lead to a rapid increase in population size (Brodie et al. 2005) and geographic spread, considering a planktonic larval duration (PLD) ranging between 9 to 42 days (reviewed in Caballes & Pratchett 2014). Different variables, such as an enhanced food supply (Brodie et al. 2005; Fabricius et al. 2010), reduced predation pressure due to overfishing (Sweatman 2008), and changes in diverse environmental variables (e.g., sea surface temperature or rainfall; Black et al. 1995; Brodie et al. 2005; Glynn 1985; Houk et al. 2007) have been postulated to increase larval or adult survival and enhance COTS outbreaks. Additional explanatory hypotheses on the local origin of outbreaks are given by changes in behaviour or survivorship of post-settlement individuals, e.g., a decrease in predation; (Endean 1969), the movement of adults between reefs (Talbot & Talbot 1971), adult aggregation (Dana et al. 1972), or outbreak cycles controlled by increase in pathogen transmission under high densities (reviewed in Pratchett et al. 2014).

Once a local outbreak (the "primary outbreak") is ongoing, the increase in larval production and migration from this primary source can lead to so called "secondary outbreaks" in downstream localities (Brodie et al. 2005; Kenchington 1997). The hypothesis of secondary outbreaks implies the transportation of a large number of larvae by oceanic currents to other localities during their planktonic stages, which would result in connectivity between distant populations across the Indo-Pacific (Kinlan et al. 2005; Scheltema 1986; Shanks et al. 2003; Treml et al. 2008). However, it is fundamental to understand the contemporary patterns of dispersion between populations to test for the existence of contemporary long-distance connectivity among COTS populations.

Considering the microscopic size of COTS larvae, analyses of its population dynamics to understand the origin of outbreaks have focused on indirect molecular methods. This approach is grounded on the assumption that organisms with short planktonic stages and low spatial dispersal capabilities have higher population genetic structure (resulting from lower levels of gene flow) than those with longer planktonic stages, which are thought to have higher levels of gene flow. A correlation between the potential for migration and genetic structure has been observed in different marine groups, including other starfish (*Linckia laevigata*; Benzie 1999b), and different species of corals (e.g. Ayre & Hughes 2000; Nishikawa et al. 2003). In the case of *A. 'planci',* a species with a long-lived planktonic larval stage (Birkeland & Lucas 1990; Caballes & Pratchett 2014), reduced genetic structure and high migration rates have been assumed (Benzie 1999a).

Although initial studies using allozymes to investigate COTS population genetics seemed to provide evidence of strong gene flow and lack of genetic structure (Benzie 1999a; Benzie & Stoddart 1992; Nash et al. 1988; Nishida & Lucas 1988), more recent analyses using different molecular markers have pointed towards a different scenario. Using the mitochondrial control region (Timmers et al. 2012; Vogler et al. 2013), internal genetic differentiation was observed within at least three of the four different clades (species) of *A. 'planci'* (i.e. the Pacific, the Northern and the Southern Indian Ocean clades) proposed by Vogler et al. (2008). Vogler et al. (2013) found support for at least four genetic groups in the Pacific Ocean and Timmers et al. (2012) discovered reduced gene flow among regions and archipelagos and significant genetic differentiation between COTS populations from the Central Pacific Ocean. Thus COTS dispersion, and thereby secondary outbreaks, seem to be limited to smaller geographic

areas, for instance within the Great Barrier Reef (GBR) (Benzie & Stoddart 1992; Benzie & Wakeford 1997), in the Ryukus Islands (Yasuda et al. 2009) and along the Hawaiian Archipelago (Timmers et al. 2011).

Although mitochondrial markers show genetic differentiation between populations with large scale geographic structure (Timmers et al. 2012; Vogler et al. 2013), these markers have not allowed to differentiate between historical evolutionary migration (dispersal) and contemporary gene flow (dispersion). For example, Timmers et al. (2012) showed that there are shared mitochondrial haplotypes between the South Central and Northwest Pacific and their haplotypes do not strictly cluster according to geographic region. This pattern was interpreted as either recent gene flow, the retention of ancestral polymorphisms or ancestral gene flow (Timmers et al. 2012). Similarly, Vogler et al. (2013) found a large geographic cluster of Western Pacific localities, with shared haplotypes in the whole range from the GBR to the Philippines. However, microsatellite data (Yasuda et al. 2009) show that significant genetic differentiation in this region is more pronounced, with patterns of isolation by distance and significant pairwise *F*st values (fixation index) between several localities indicating intra-cluster genetic differentiation.

The differentiation between contemporary genetic patterns and evolutionary history is of importance for conservation biology as highlighted by several authors (e.g., Peijnenburg et al., 2006, Selkoe and Toonen, 2006, Eytan and Hellberg, 2010, van der Meer et al., 2012). The mutation rate of mitochondrial DNA is known to be suitable to resolve taxonomic uncertainties and historical biogeographical events, but it may not be suitable to infer contemporary migration events (Wan et al, 2004). Microsatellites, which evolve up to 100 times faster than mitochondrial DNA, provide enough variance for inferring contemporary patterns of gene flow (Wan et al, 2004) and are more suitable to study the connectivity of recent COTS outbreaks. Despite their many advantages, there are only two studies using microsatellites from COTS and they are mainly concerned in the connectivity patterns among Western Pacific populations (Yasuda *et al.* 2009) and locally at the Society Islands, French Polynesia (Yasuda et al. 2015). Contemporary connectivity on a broad geographical scale in the Indo-Pacific has not been tested yet.

This study aims to investigate the contemporary genetic structure of the Pacific crown-of-thorns starfish species using microsatellites and to test for isolation among distant geographical regions previously identified as a cohesive genetic unit by mitochondrial DNA. We investigated genetic patterns of connectivity in the context of the COTS outbreaks in Guam. We compared samples from around Guam with a number of distant localities in the Pacific Ocean, and focused on determining contemporary gene flow between populations previously considered to be isolated (i.e. Johnston Atoll, Kingman Reef, Swains, Japan, the Great Barrier Reef , Vanuatu, Moorea, and Philippines).

Materials and Methods

Sampling

Guam is the largest and most southern island of the Marianas archipelago. It is located in the Western Pacific Ocean within Micronesia. The impact of *A. 'planci'* on this island has been reported since the early 1970's (Gawel 1999), and surveys from 2003 to 2007 found numerous outbreaks in different coral reefs around the island and detected an increase in outbreak intensity in each subsequent expedition (Burdick et al. 2008). A total of 274 *A. 'planci'* tissue samples from pyloric caeca and tube feet were collected by SCUBA diving or snorkelling; 172 individuals were collected from six localities around Guam in 2006 and 102 individuals were from nine other reef localities in the Pacific (Figure 1 and Table 1). No collection permit was needed for the conducted research as no COTS were collected from designated Marine Protected Areas around Guam. Five of the sampled localities around Guam had densities of *A. 'planci'* with more than 150 COTS per hectare, with Tanguisson Reef having the highest density of 522 COTS per hectare. Only one locality, Taguan point (G3), was considered to be a non-outbreak population (< 30 COTS per hectare). The sampling outside Guam was designed based on previous studies using sequences of the mitochondrial control region to include localities from the most distinguishable genetic groups in the Pacific (based on Vogler et al. 2013) around Guam, named West, North-Central, North-West and South-Central Pacific. Since the goal of this study was to evaluate the contribution of long distance migration, the sampling included islands located more than 2000 km away from Guam. For example, there are about 2400 km straight line distance from Guam to the Philippines and 2500 km from Guam to the south of Japan. Additionally, in the

same year of 2006, massive outbreaks were observed on some of the islands upstream from Guam on the major currents, including Kingman Reef in the eastern central Pacific and Swains Island in the south Pacific, making them possible long distance COTS sources (distances of ca. 5800 km and 5600 km respectively). Although genetic connectivity has not been reported between some of the sampled localities, e.g., Johnston Atoll and Guam, and oceanic currents predict isolation, these localities were also included here to characterise the variance of the markers used, control for possible homoplasy or as an internal methodological control, assuming that those populations should be genetically highly differentiated.

The samples were stored in ethanol 80% or DMSO buffer at -80°C. A MagAttract 95 DNA Plant Core Kit (Qiagen) was used to extract total DNA from tube feet and pyloric caeca samples, following the recommended protocol by the manufacturer. As a preliminary step, the tissue was ground after freezing in liquid nitrogen, and incubated for 1 hour at 35°C in RLT lysis buffer (Qiagen). In the case of tube feet, DNA was extracted using the DNeasy Tissue Kit (Qiagen), according to the protocol recommended by the manufacturer.

All samples were genotyped using a set of 13 microsatellites previously identified for *A. 'planci'* (Yasuda et al. 2006; Yasuda et al. 2007). The set included the loci Yukina01, Yukina05, Yukina06, Yukina08, Maki01, Maki03, Tama01 and Hisayo01 from Yasuda et al. (2006) and Aya02, Maki12, Maki11, Tama11 and AyU03 from Yasuda et al. (2007). Standard three-step PCR reactions were conducted for each locus in a final volume of 12.5 µl of GoTaq Flexi Buffer® 1x, $MgCl_2$ 3mM, dNTPs 0.4 mM, primers forward and reverse 0.2 µM, BSA 0.08 mg/ml and 0.5u of GoTaq® polimerase (Promega) with 1 µl of DNA template (around 20 ng of DNA). For fragment length analysis, the 5 'end of the forward primers used in the PCR were labelled with a fluorescent dye (HEX, 6-FAM or BoTMR). The PCR cycling conditions were as follows: 10 min at 94°C, 38 cycles of 30 s at 94°C, 30 s at 56-60°C (primer-specific annealing temperature), and 1 min at 72°C, and a final elongation of 5 min at 72°C.

PCR products were mixed for genotyping in 3 different co-loading reactions as follows: co-loading 1 included loci Yukina01, Yukina05, Yukina06 and Yukina08; co-loading 2 included Maki01, Maki03, Tama01 and Hisayo01; and co-loading 3 included Aya02, Maki12, Maki11, Tama11 and AyU03. Samples were analysed on an ABI 3730 48

capillary sequencer (Applied Biosystems) using the dye set D and G5 and 400HD ROX size standard at the Sequencing Service of the Department of Biology at the Ludwig-Maximilians-Universität in Munich (Germany). The software GeneMaper® v.4.1 was used to call allele sizes.

Data Analysis

A Markov chain algorithm implemented in the software GENEPOP v.4.2 (Raymond & Rousset 1995; Rousset 2008) was used to test each locus per location for departure from Hardy-Weinberg equilibrium (HWE). The same software was used to assess linkage disequilibrium (LD) between different combinations of paired loci. The analysis of HWE was conducted with a dememorisation period of 10000 generations, 100 batches and 5000 iterations per batch. In the case of LD, the number of batches was increased to 1000. Additionally, the software Micro-Checker v.2.2.3 (van Oosterhout et al. 2006) was used to test for systematic distortion of HWE in each locus, which is an indication for the presence of null alleles, large allele dropout or other scoring errors. Sequential Bonferroni corrections for multiple comparisons were used to adjust the threshold of statistical significance in both analyses (Holm 1979; Rice 1989). Loci that departed from HWE, showed LD or evidence of errors in scoring were not included in subsequent analyses.

Genetic diversity within each locality was determined through the estimation of number of alleles per locus and locality, gene diversity, observed and expected heterozygosity and allelic richness using the software ARLEQUIN v.3.5.1.2 (Excoffier & Lischer 2010) and GENEPOP v.4.2 (Raymond & Rousset 1995; Rousset 2008). The permutation of localities (1000 randomizations) was used to determine differences in genetic diversity using the software FSTAT v.2.9.3.2 (Goudet 2001).

A hierarchical analysis of molecular variance (AMOVA) loci by loci was carried out in ARLEQUIN v.3.5.1.2 (Excoffier et al. 1992; Excoffier & Lischer 2010). AMOVA (with 20,000 permutations) was used to determine genetic diversity (as a source of covariance) and its significance within localities, between localities, between islands. The AMOVA analysis was also performed by grouping islands according to their predicted connectivity based on oceanic currents and passive larval dispersion (Treml et al. 2008), and also grouping them based on clusters obtained from the analysis using the program STRUCTURE (see below). The purpose of this last analysis was to evaluate

the strength of the separation between the inferred clusters. Population pairwise *Fst* values were estimated and significance was assessed using 20,000 permutations and Bonferroni correction. Confidence intervals were estimated with the package *diveRsity* (Keenan et al. 2013).

The estimation of the number of distinct populations and the assignment of individual samples to populations was done using the software STRUCTURE v.2.3.4 (Falush et al. 2003; Falush et al. 2007; Hubisz et al. 2009; Pritchard et al. 2000). The number of potential populations or clusters (K) was evaluated using values for K from 1 to 10, with at least 12 independent runs for each value. Uniform priors in an admixture ancestry model were used in each run with a burn-in period of 200,000 generations, a posterior sampling chain of 1,000,000 generations and the assumption of correlated allele frequencies among samples. The determination of the most accurate value for K was evaluated using the statistic ΔK following the methodology of Evanno et al. (2005).

Finally, contemporary gene flow between populations was also determined by a discriminant analysis of principal components (DAPC; Jombart et al. 2010). This last analysis was performed using the package *adegenet* (Jombart et al. 2010) implemented in R v.3.0.1 (http://www.r-project.org/). Individuals with missing data were not included in this analysis.

Results

Hardy-Weinberg Equilibrium, Linkage disequilibrium and possible genotyping errors

Based on an exact test, using a Markov chain for each locus per sampled location, three loci deviated significantly from HWE in almost all locations (initial p-value < 0.05 after sequential Bonferroni correction). These loci were *Tama01, Maki11* and *Tama11,* which deviated in more than 8 (out of 14) locations. Additionally, the analysis with Micro-Checker showed a consistent excess of homozygotes in the same markers, suggesting the presence of null alleles, polymerase stuttering or large allele dropout. Because these results were consistently bias for most of the sampled localities, those three loci were not included in subsequent analyses.

The remaining markers showed HWE, with punctual deviations in some populations (i.e. *Yukina05, Mak03, Aya2* and *AyU03* in non-HWE in 2, 1, 3 and 4 localities, respectively). In these cases the tests used did not show evidence of linkage disequilibrium, null alleles, or biases in the identification of genotypes. Thus, these loci were included in all subsequent analyses.

Gene diversity within populations

Most of the microsatellite loci were highly polymorphic. Two exceptions were found: *Yukina08* in Johnston Atoll and *Maki12* in Moorea and Johnston Atoll, where only one allele was fixed in the populations. These two markers, as well as *Aya2*, showed the lowest number of alleles (6 to 8) for the entire set of samples and the lowest number of alleles per location (1 to 6) (Table 1 and Supplementary Table S1). On the other hand, the rest of the markers were highly polymorphic with a total number of alleles ranging between 9 and 18, with 2 to 14 alleles per locus per locality.

The high genetic diversity suggested by the number of alleles in each location contrasted with the findings of allelic richness. The high genetic variability within localities precluded observing differences in allelic richness between localities (Supplementary Table S1). After correction for different sampling sizes using a rarefaction analysis, the lowest value of allelic richness was found at Johnston Atoll (2.85), the highest values were observed for the localities in Guam (around 5.11), Japan (5.20) and the GBR (5.22). Despite these differences, the allelic accumulation function, which predicts the expected number of alleles to be observed if the localities would have had the same sample size (Van Loon et al. 2007), did not show differences in allelic richness between localities due to a broad confidence interval (Supplementary Figure 1).

Genetic structure

The range of pairwise *Fst* values obtained ranged from 0.000 to 0.480 (Table 2). In general, the lowest values were found between localities inside Guam and the highest in most comparisons involving Johnston Atoll. *Fst* analysis showed three significantly differentiated regions composed by (1) all the localities immediately around Guam and Kingman as well as the Swains Islands, (2) the GBR, Philippines, Japan and Moorea and (3) Johnston Atoll, which in addition was significantly different from all other

localities. Pairwise *Fst* values between localities inside regional groups were significantly lower (around 0.05 as the highest value) than between localities from different regional group (*Fst* values higher than 0.15) (Table 2, confidence intervals in Supplementary table S2).

Considering the first group of localities, it is important to note that all the localities around Guam, except Tipalao Bay (G1), had statistically significant genetic similarities with Kingman Reef and/or Swains Islands. This is noteworthy because the population in Tipalao Bay was the only aggregation in the southwest coast of Guam during surveys and COTS were almost absent in adjacent reefs (Caballes pers. obs.). Moreover, the *Fst* values obtained for several of the comparisons between Guam populations were higher than those obtained in comparisons between Kingman or Swains and localities around Guam. For example, the comparison between North Haputo point (G4) and Tagachan point (G6), and Tipalao Bay (G1) and Taguan Point (G3) resulted in *Fst* values of 0.034 and 0.032, respectively. In contrast, the comparison between Kingman Island (K) and Taguan Point (G3) in Guam had a *Fst* of 0.006 and the value between the comparison between Swains Island (S) and Urunao Point (G2) was 0.010. However, the genetic differences observed among localities around Guam and between Guam and Kingman or Swains Island were not statistically significant given the broad confidence intervals of the *Fst* values.

According to the results of the AMOVA, the percentage of genetic covariance explained by individual variation was 84% (Variation within localities in Table 3). 15% of the genetic variation can be attributed to differentiation between islands and only 1% is explained by variation among localities (i.e., sampling sites). The same analysis grouping islands according to the three groups found in the pairwise *Fst* analysis and in Bayesian clustering with STRUCTURE (see below) resulted in a reduction in the percentage of genetic variance explained by individual variation (Variation within localities = 80% in case 4 and 5 from Table 3) and in an increase in the percentage of variance explained by regions (18%). Grouping islands based on oceanic currents (Treml et al. 2008) also increased the variance explained by regions (14 and 17% in case 2 and 3 from table 3) and *Fst* values, but the values were lower than in the previous two analyses (case 4 and 5 from table 3). .

Population structure

The Bayesian clustering analysis with STRUCTURE and the estimation of ΔK (Evanno et al. 2005) indicated the existence of two peaks in the most likely number of ancestral gene pools. The highest value of ΔK was obtained for K = 2. A second peak was observed at K = 4. This last value coincides with the point in which there is a significant change in the slope of the likelihood distribution. Moreover, the analysis of DAPC showed a significantly low Bayesian Information Criterion (BIC) value for the existence of 5 genetically different groups (Figure 2 and Supplementary Figure 2).

When the samples were assigned to two genetic groups (K = 2), Guam, Kingman Reef and Swains Islands clustered together and were significantly different from all other locations sampled in this study (Figure 2). When the number of genetic groups was increased to four and five, the initial differentiation in two groups was maintained with additional information within each group. First, mixing between Guam, Kingman and Swains was evident, with a different proportion of individuals belonging to each predicted population inside localities (represented as blue and red colours in the bar plot with K = 4, and blue, red and yellow in the plot with K = 5). In the second group, the change in the number of predicted populations from 2 to 5 revealed the differentiation of Islands such as Moorea and Johnston Atoll, and increased the heterogeneity observed inside the GBR.

Migration and recent gene flow

The first two components of the DAPC explained 41.5% and 40.7% of the variation in the dataset and results were consistent with the results found with STRUCTURE (Figure 3A). In this analysis, the level of genetic similarity, possibly explained by gene flow, was represented by a clustering of the genotypes of each locality. Including all localities in the analysis, Johnston Atoll stood out as a strongly divergent group, possibly isolated and without gene flow to/from the two main groups.

Because the genetic differentiation of Johnston Atoll with the other islands was strong, additional DAPC analyses were performed (Figure 3B and C). Aiming to gain insights into the genetic differentiation within each group, in one analysis Johnston Atoll was excluded and in a second one only localities from Guam, Kingman and Swain Islands were included. Excluding Johnston Atoll, the level of clustering between localities was more pronounced for the first group (Guam, Kingman and Swains) than the second group (GBR, Japan, Philippines and Vanuatu). This is of considerable interest, because

geographic distance between Guam, Kingman, and Swains was higher compared to distances between localities in the second group. Additionally, although gene flow between all the locations of the second group seemed evident, there was an apparent subdivision inside the group with the GBR grouping with Vanuatu and Japan grouping together with the Philippines (Figure 3B). The differentiation between these two subgroups was given only by the second axis (Y axis) of the DAPC and the percentage of variance explained by this axis was low (6.4% of the variance).

Localities within Guam (Figure 3C) showed higher levels of genetic similarity. However, it is important to observe that this genetic similarity is variable, with some localities in Guam more related to Kingman (for example G3, G4, G5 and G6) and with a slight overlap with Swains and other localities more isolated from Kingman and Swains (locality G1). These results are evidence for the existence of genotypes in Guam that are different to those found in Kingman and Swains Islands (unique haplotypes).

Discussion

Contemporary long distance dispersion across the Pacific

This study suggests long distance dispersion of *A. 'planci'* between localities in the Pacific and genetic structure within large geographical regions in this basin. The sampled localities in the Pacific were found to be structured in at least three large groups with apparent limited larval dispersion between them. The first group comprised Guam, Kingman Reef and Swains Islands; the second group included the GBR, Vanuatu, Japan, and Philippines; while Johnston Atoll was in the third group. Although a general high genetic diversity was found inside each sampled island, our analyses showed strong genetic similarities between the localities of Guam, Kingman and Swains Islands suggesting recent larval dispersion between these geographically distant regions. Such a pattern has also been observed in other marine organisms with high larval dispersal potential in the Pacific Ocean, mainly using allozymes (e.g. *Linckia laevigata,* Williams & Benzie 1997; some species of corals, Ayre & Hughes 2000; sea cucumbers, Uthicke & Benzie 2003).

Connectivity of marine organisms has been modelled as a diffusion process in which larvae and juveniles are transported by oceanic currents between suitable habitats

(Treml et al. 2008; Kool et al. 2011; Treml et al. 2012; Wood et al. 2014; Treml et al. 2015). In these models, one of the criteria determining the connectivity between localities is the duration of the larval stage (planktonic larval duration, PLD) — assuming that organisms with longer PLDs are capable of migrating longer distances. According to these models, the dispersal potential of *A. 'planci',* with a long PLD of up to 42 days (reviewed in Pratchett et al. 2014), would allow the migration of individuals across long distances, potentially connecting the islands of Guam, Kingman and Swain. Our results are in agreement with this prediction, because our microsatellite data suggested genetic connectivity between Guam, Kingman and Swain islands.

Long distance connectivity is especially important during events like *El Niño* (Treml et al. 2008), when some current systems in the Pacific deviate from established patterns. Treml et al. (2008) suggested that for corals, Pacific-wide connectivity is strongly reduced when a probability of successful dispersal of 0.5 is selected (Treml et al. 2008). The strong genetic differentiation observed between Guam and other Western Pacific localities, namely Philippines and Japan, can be interpreted as a westward drop in larval dispersion, likely caused by the reduced strength of the oceanic currents flowing East-West and the lack of stepping stones between these localities. This combination would make East-West larval dispersion difficult even for organisms with long PLDs (Treml et al. 2008; Kool et al. 2011; Treml et al. 2015).

There are important discrepancies between our study and previous studies using the control region of the mtDNA (Timmers et al. 2012; Vogler et al. 2013) regarding population structure and connectivity between localities in the Pacific Ocean. According to Timmers et al. (2012), populations in the Central Pacific are genetically differentiated into three main regions: North, South and North-West Pacific. They found that the Johnston Island is part of the North region; Kingman, Swains and Moorea Islands belonged to the South region and Guam to the North-West region. Additionally, Vogler et al. (2013) grouped Guam in a large western region with Japan, Philippines and the GBR. Our results, using microsatellites, agree with the control region mtDNA data in the broad geographical zonation in the Pacific, but contrary to mtDNA data, this study is consistent with a new hypothesis of larval dispersion between the south-central Pacific (Kingman and Swains) and the north-west Pacific (Guam), as suggested by the strong genetic similarity between Guam, Kingman and Swains Islands.

Discordances between mtDNA and microsatellites data have been already reported in *A. 'planci'* populations from the Pacific Ocean. Using microsatellites, Yasuda et al. (2009) found that *A. 'planci'* populations in the western Pacific (i.e., Japan and Philippines), the GBR, and the North Pacific Islands (i.e. Palau, Majuro and Pohnpei, which are geographically close to Guam) belonged to different genetic groups. In contrast, when using mtDNA control region, Vogler et al. (2013) found that *A. 'planci'* samples from Palau were closer to the western Pacific population, while Majuro and Pohnpei were more related to the GBR.

The most plausible explanation for this discrepancy can be differences in the marker's potential to resolve different time- and spatial scales. Several authors have highlighted the importance to distinguish between recent contemporary events and evolutionary history (e.g., Eytan & Hellberg 2010; Peijnenburg et al. 2006; Selkoe & Toonen 2006; van der Meer et al. 2012; van der Meer et al. 2013). In general, the high mutation rate of microsatellites results in rapid allelic changes in the population, thus contemporary demographic changes or recent connectivity patterns are more adequately reflected by these markers (Selkoe & Toonen 2006). In contrast, other markers with lower mutation rate, like mtDNA, could be more informative to uncover the signature of events in the more distant evolutionary history.

Our data suggest that localities like Guam, Kingman Reef and Swains Islands were likely isolated from each other in the past, but are currently connected by contemporary gene flow, a hypothesis consistent with the analysis of the mitochondrial control region (Timmers et al. 2012). These patterns of mitochondrial divergence were interpreted as the result of occasional exchange of larvae between distant areas, the retention of ancestral polymorphism or a signature of ancient gene flow. Based on our analysis of more variable markers (i.e., microsatellites), we propose that the ancestral mitochondrial divergence signature between distant localities is being gradually eroded by contemporary gene flow. In agreement with our interpretation, Vogler et al. (2013) also found evidence for a recent population expansion in a large group of Pacific populations (including Guam) and a geographical mix of divergent mitochondrial haplotypes, resulting in a star-shaped minimum spanning haplotype network.

Other several mutually non-exclusive explanations could also explain the discrepancy between control region mtDNA and microsatellite data. This includes non-neutral

evolution of mtDNA (Ballard & Whitlock 2004) with the potential for sex-biased migration or selection on specific haplotypes; differences in the effective population size, resulting in differences in the effect of genetic drift (Shaw et al. 2004); or the higher likelihood for homoplasy in microsatellites over longer periods of time, due to higher mutation rates (O'reilly et al. 2004). However, regardless of the explanation of the observed discordance among nuclear and mtDNA markers, the microsatellite data presented here suggests contemporary long distance migration as the most likely scenario.

Differences within Guam

Considering the apparent lack of genetic structure between geographically distant regions, some evidence of genetic differentiation among localities around Guam were found. For example, it was found that some localities around Guam (e.g., Tipalao Bay (G1), Urunao Point (G2) and North Haputo Point (G4)) were genetically differentiated from Kingman Reef or/and Swains Islands (considering pairwise Fst values), but other localities around the same Island (e.g. Taguan (G3) or Tanguisson(G5)) are suggesting dispersion from and to these distant localities. A similar pattern was found in the discriminant analysis of principal components (DAPC, Figure 3), however, these genetic differences were not statistically significant due to the large confidence intervals of the *Fst* values. Additional studies are needed to determine if those differences are biologically relevant or result from an increased variance inherent to the implemented methodology. Factors causing these plausible variations in larval dispersal patterns remain to be determined, especially considering that previous studies using less variable markers, such as allozymes or control region mtDNA, have identified genetic differences between local COTS populations (Benzie 1999a; Benzie & Stoddart 1992; Nash et al. 1988; Nishida & Lucas 1988; see Timmers et al. 2012 for within island differentiation).

COTS gene flow in general may be affected by a number of factors, such as different oceanographic conditions, climatic fluctuations, local adaptation and differential mortality of pre-settlement larval stages (Benzie & Stoddart 1992; Yasuda et al. 2009). Distribution and dynamics of *A. 'planci'* are sensitive to changes in food availability (abundance of coral prey), food quality (preferred coral species), and population densities (De'ath & Moran 1998; Kayal et al. 2012). The sampled coastal localities in

Guam also differ in terms of the amounts of riverine discharge and hydrodynamic patterns (Wolanski et al. 2003), and the reefs vary in coral cover and community structure (Burdick et al. 2008). Changes in local conditions linked to anthropogenic activities (e.g. increased sedimentation, terrestrial runoff and overfishing; Brodie et al. 2005) are capable of triggering primary outbreaks and may also facilitate larval settlement and survival, leading to increased adult numbers and secondary outbreaks. All these risk factors have increased in the last decades in Guam (Burdick et al. 2008; Gawel 1999), which may help to explain the increase in the frequency and impact of *A. 'planci'* in this island.

Conclusions

In this study, genetic connectivity of the crown-of-thorns starfish (*Acanthaster 'planci'*) around Guam was evaluated using microsatellites and compared to spatially distinct localities in the Pacific. Genetic structure was detected within the sampled Pacific localities, which suggests clustering of reefs into broad geographic groups. However, these groupings are not consistent with previous findings based on the control region of the mtDNA. Gene flow was observed between Guam Island and distant reefs, such as Kingman and Swains, previously considered isolated regions. This finding highlights the necessity of studying the origin and development of COTS outbreaks using highly variable genetic markers that provide enough variance to infer contemporary patterns of gene flow, instead of evolutionary/biogeographic histories. Additional studies are needed to address the strength of the long-distance dispersion events and extent and frequency of secondary outbreaks and ideally should include a spatially more dense sampling. Our findings are relevant for coral reef conservation, as they emphasize the importance of identifying the triggers of primary and secondary outbreaks and the need for the development of risk management strategies and control programs to reduce the impact of COTS on coral reefs. Our results also highlight the need to address the COTS problem at both regional (Pacific Ocean) and local (within islands) scales.

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FIGURES AND TABLES

Figure 1. A: *Acanthaster 'planci'* localities sampled in the Pacific Ocean. Localities are coded by geographical regions: west Pacific (W), north-west Pacific (NW), north-central Pacific (NC), south central Pacific (SC). GBR represents the Great Barrier Reef. Current paths are presented in dashed line: North Equatorial Countercurrent (NECC), North Equatorial Current (NEC), South Equatorial Countercurrent (SECC), and South Equatorial Current (SEC). **B:** Details of sampling locations around Guam. Maps are taken and modified from www.arcgis.com.

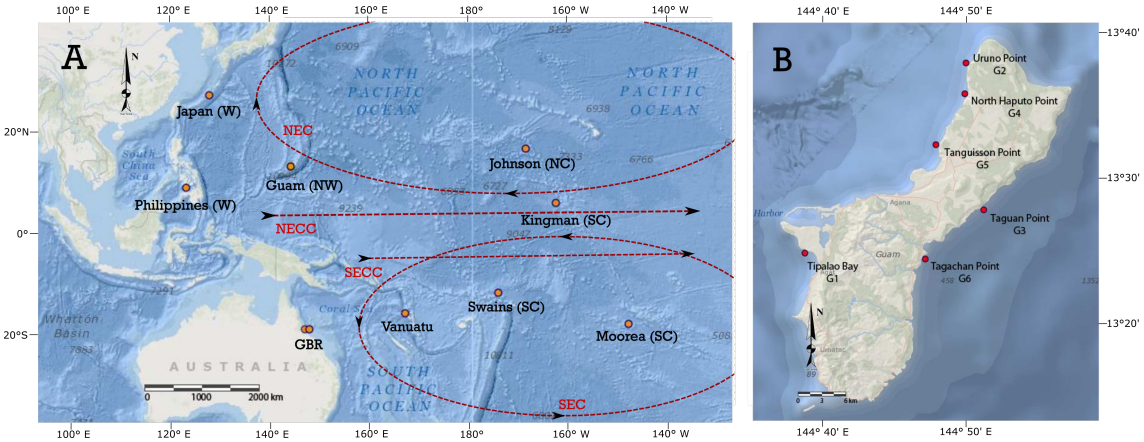

Figure 2. Graphical summary of Bayesian clustering results. Samples were assigned among 2, 4 and 5 genetic clusters (K). Each colour represents the probability of corresponding to a specific cluster. Each locality is separated by a black line. The Guam group (Guam, Kingman and Swains Islands) is highlighted with the black line.

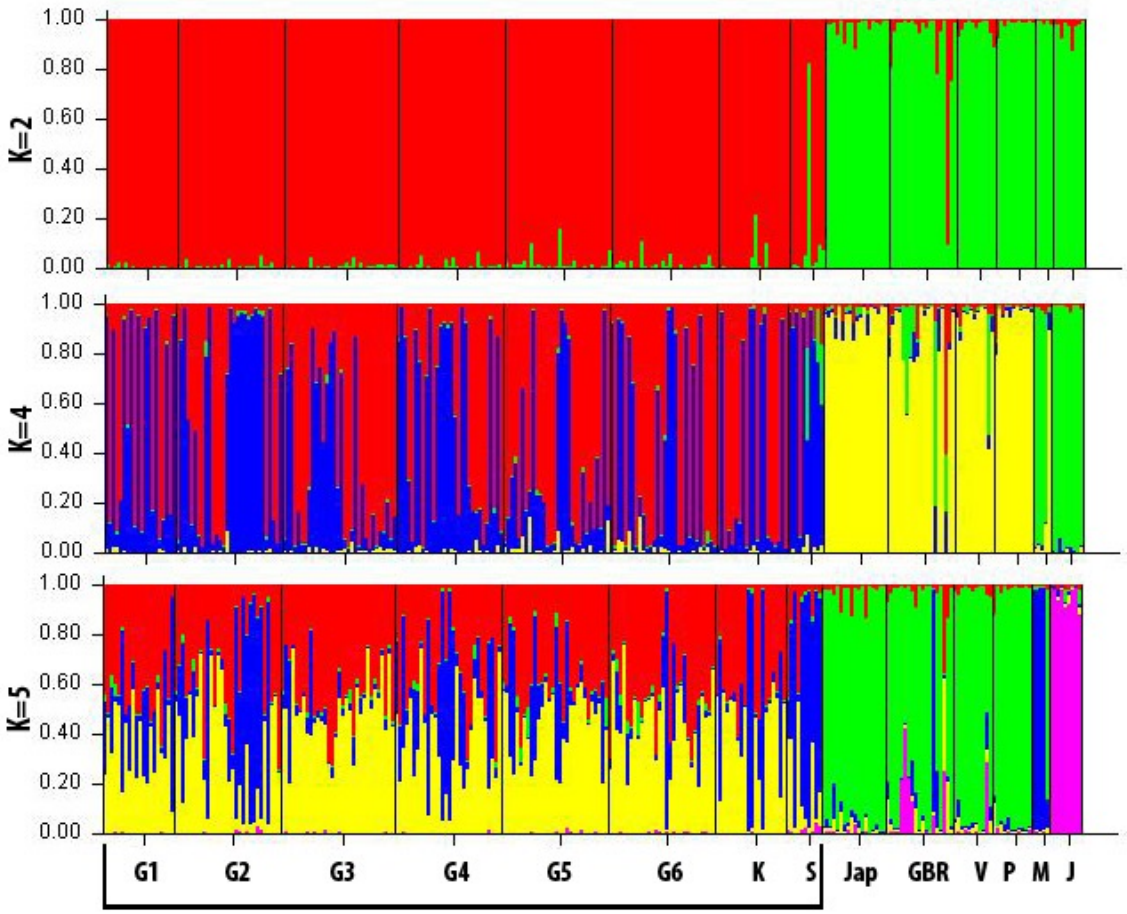

Figure 3. Scatterplots of the discriminant analysis of principal components (DAPC) for all localities (A), group 1 (Guam, Kingman and Swains Islands) and 2 (GBR, Japan, Philippines, Vanuatu) (B) and only the group 1 (C). Individual genotypes appear as dots surrounded by 95% inertia ellipses. Eigenvalues show the amount of genetic information contained in each successive principal component with x- and y-axes constituting the first two principle components, respectively.

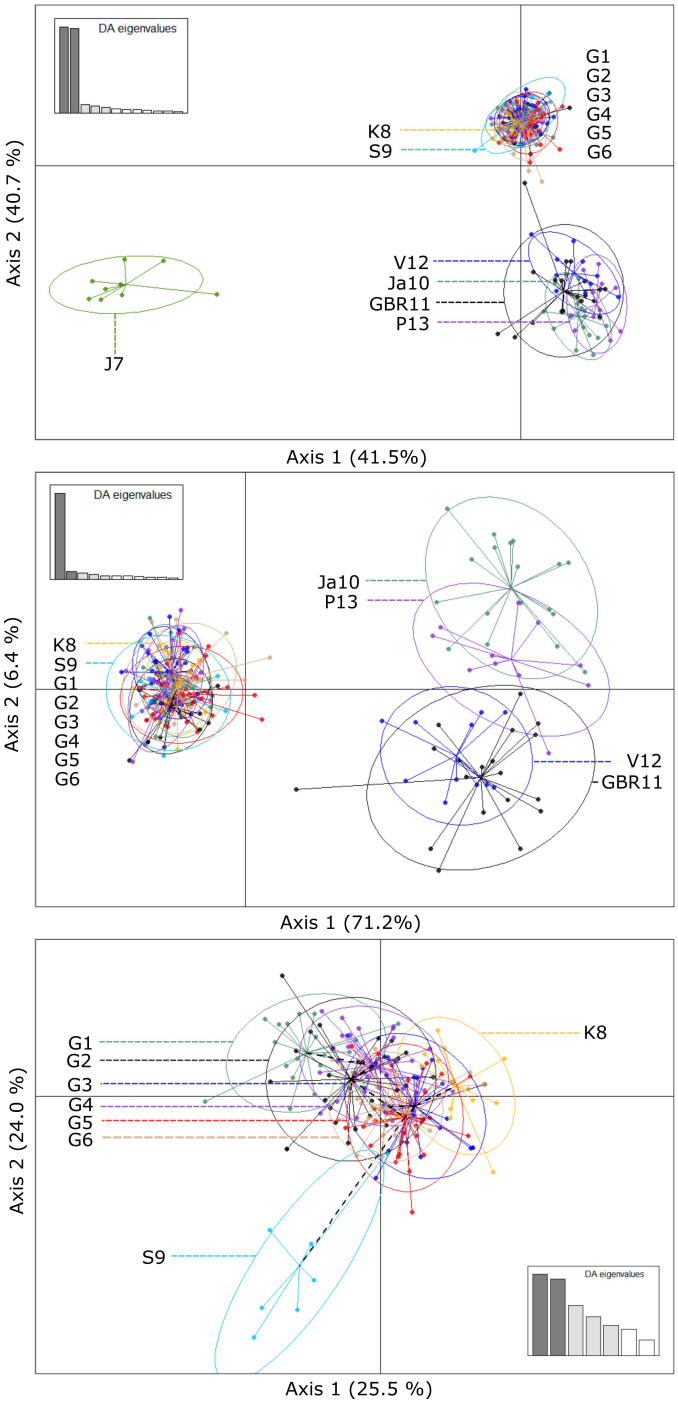

Table 1. Summary information of sampled localities, code of locality, collection year, number of samples (N), number of alleles (Na), allelic richness (Ar), observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficient (Fis).

Sample Location	Code	Colection Year	N	Na	Ar	Ho	He	Fis	test P-value
Tipalao, Guam	G1	2006	20	6,9	5,01	0,6708	0,6896	0,028	0,593
Uruno, Guam	G2	2006	30	8,0	5,42	0,6745	0,7361	0,085	0,022
Taguan, Guam	G3	2006	32	7,3	5,04	0,6987	0,7047	0,009	0,171
North Haputo, Guam	G4	2006	30	7,5	5,14	0,6533	0,7008	0,069	0,015
Tanguisson, Guam	G5	2006	30	7,3	5,00	0,6900	0,7176	0,039	0,185
Tagachan, Guam	G6	2006	30	7,3	5,07	0,6467	0,7055	0,085	0,184
Johnston Atoll	J	2006	9	3,1	2,85	0,3333	0,4575	0,284	0,053
Kingmanreef	K	2006	20	5,5	4,37	0,5833	0,6704	0,133	0,004
Swains	S	2006	10	5,4	4,87	0,6643	0,7228	0,085	0,042
Japan	Ja		18	6,8	5,02	0,4923	0,6869	0,290	0,000
GBR	GBR	1999	19	7,0	5,22	0,3458	0,7442	0,542	0,000
Valuatu	V		11	5,0	4,52	0,3685	0,7101	0,494	0,000
Moorea	M	2006	5	3,3		0,3556	0,6173	0,617	0,006
Phillipines	P		11	5,4	4,70	0,2795	0,6558	0,588	0,000

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Table 2. Pairwise FST for 13 *Acanthaster 'planci'* localities. Bold numbers indicate statistical significance after Bonferroni correction at P < 0.05. The red and blue squares show regional groups found in this study.

	G1	G2	G3	G4	G5	G6	K7	S8	Ja9	GBR10	V11	P12	J13
G1	0,000												
G2	0,003	0,000											
G3	**0,032**	0,015	0,000										
G4	0,008	-0,002	**0,025**	0,000									
G5	0,019	0,007	0,000	0,009	0,000								
G6	**0,026**	0,015	-0,004	**0,034**	0,007	0,000							
K7	**0,048**	**0,032**	0,006	**0,039**	0,011	0,018	0,000						
S8	**0,045**	0,010	0,024	0,021	0,021	0,027	0,039	0,000					
Ja9	**0,235**	**0,208**	**0,207**	**0,222**	**0,192**	**0,200**	**0,229**	**0,200**	0,000				
GBR10	**0,184**	**0,155**	**0,164**	**0,169**	**0,149**	**0,167**	**0,174**	**0,147**	0,050	0,000			
V11	**0,189**	**0,148**	**0,160**	**0,168**	**0,143**	**0,155**	**0,184**	**0,157**	0,047	0,037	0,000		
P12	**0,257**	**0,218**	**0,214**	**0,235**	**0,201**	**0,213**	**0,226**	**0,225**	0,027	0,062	0,051	0,000	
J13	**0,335**	**0,295**	**0,313**	**0,323**	**0,322**	**0,313**	**0,354**	**0,328**	**0,446**	**0,373**	**0,410**	**0,480**	0,000

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Table 3. Results from AMOVA grouping localities by islands and regional groups. In all cases p-values were highly significant (<0.001). SS: sum of squares; VC: variance component; PV: percentage of covariance. In all cases all localities were included in the analysis, but the regional groups change. Groups in case 2 and 3 are based on models using oceanic currents and different PLB (30 and 60 days) (Treml et al. 2008). Groups in case 4 and 5 are based on finding from Bayesian analyses.

Factor	Islands	Localities within islands	Within localities	Total	Regions	Localities within regions	Within localities	Total	Regions	Localities within regions	Within localities	Total	Regions	Localities within regions	Within localities	Total	Regions	Localities within regions	Within localities	Total
SS	216,80	29,47	1790,13	2036,39	208,16	38,11	1790,13	2036,39	127,67	118,60	1790,13	2036,39	175,03	71,24	1790,13	2036,39	201,32	44,95	1790,13	2036,39
VC	0,62	0,04	3,48	4,14	0,61	0,06	3,48	4,14	0,77	0,22	3,48	4,46	0,80	0,09	3,48	4,36	0,79	0,06	3,48	4,33
PV	15,02	1,03	83,95	100,00	14,65	1,35	84,00	100,00	17,17	4,84	77,99	100,00	18,34	1,97	79,69	100,00	18,34	1,38	80,28	100,00
FST				0,160				0,160				0,220				0,203				0,197
FSC				0,012				0,016				0,058				0,024				0,017
FCT				0,150				0,147				0,172				0,183				0,183

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