# LIMITED GENETIC STRUCTURE AMONG POPULATIONS OF THE RED PENCIL URCHIN *HETEROCHENTROTUS MAMMILLATUS* (L.) FROM HAWAIʻI AND KINGMAN REEF

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF ARTS

IN

GEOGRAPHY

DECEMBER 2011

By
Holly Bolick

Thesis Committee:

Stacy Jorgensen, Chairperson
Shelley James
James Maragos
Robert Toonen

## ACKNOWLEDGEMENTS

I would like to thank Papahānaumokuākea Marine National Monument, the U.S. Fish and Wildlife Service, and Hawai‘i Division of Aquatic Resources for permission to perform research activities and assistance with permits, and the U.S. National Oceanic and Atmospheric Administration research vessel Hi‘ialakai and her crew for logistical support. Special thanks to Daniel Wagner, Molly Timmers, Ken Longenecker, Hawaii Institute of Marine Biology (HIMB) employees and members of the ToBo Lab for assistance with the collection of the samples**.** I would also like to thank Kim Andrews for sequencing the samples for the COI gene at HIMB, Shepherd Meyers for help with the illustrations of the sample and distribution maps, Melanie Tengan for initial sample processing assistance, Derek Skillings for the use of his COI primers, and the HIMB EPSCoR Core Genetics Facility and the University of Hawaii Genetics Facility in the Department of Microbiology at the University of Hawai‘i at Mānoa. This research was funded by Shelley James at the Bishop Museum Pacific Center for Molecular Biology, Stacy Jorgensen in the Department of Geography at the University of Hawai‘i at Mānoa, and Rob Toonen of HIMB. Thanks also to James Maragos and others for help with feedback and final editing of the document.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## ABSTRACT

*Heterocentrotus mammillatus*, the red pencil sea urchin, is a mass spawning benthic marine species with a large tropical Pacific distribution that is native to the State of Hawaii, which includes one of the largest marine protected areas in the world. Geographic patterns of genetic diversity in the red pencil urchin were investigated within its northeastern range in order to help understand dispersal patterns of marine species throughout the area, and to provide additional (species genetic population structure and distribution) information to assist with coral reef management in and around Hawaii's extensive marine protected areas. Two genetic techniques were used for this study: the mitochondrial DNA region cytochrome oxidase subunit I (COI) and genome-wide amplified fragment length polymorphism (AFLP) markers. Seven populations were sampled within the study area: three populations each from the main and northwest Hawaiian Islands, and an additional population from Kingman Reef (Line Islands) located approximately 1500 km south of the main Hawaiian islands. Both analyses produced concordant results and found that the vast majority of genetic diversity was within populations (99% for COI and 96% for AFLP). Of the 42 total COI haplotypes identified, 28 were found in a single population, three of the most common haplotypes were shared by all populations, and no strong geographic trends were observed in the haplotype network. Average COI haplotype diversity was high at 0.85 and nucleotide diversity was relatively low at 0.01. Of the 146 AFLP loci, 121 were polymorphic in at least one population, and the average expected heterozygosity for all seven populations together was 0.418 (with a range from 0.256 to 0.428). While an analysis of molecular variance (AMOVA) of the COI data identified significant genetic structure between

Hawaiian populations and Kingman Reef (3% total variation), the AMOVA of the AFLP data was able to identify significant genetic substructuring at three spatial scales: among populations within the main and northwestern Hawaiian islands (3% total variation), between the main and northwestern islands (1.2% variation), and between Hawaii and Kingman Reef (5% total variation). Within the Hawaiian Archipelago, however, there was no evidence of isolation-by-distance for either analysis, indicating a chaotic or patchy pattern of gene flow among populations.

# CHAPTER 1: INTRODUCTION

The pathways of dispersal in highly vagile larvae of benthic marine fauna remain poorly understood. For example, limitations to larval dispersal throughout the ocean are not easily identifiable due to the fluid nature of the sea and the lack of obvious visual barriers for dispersal (Lessios *et al.* 1998; Galindo *et al.* 2006; Baums & Paris 2006; Fogarty & Botsford 2007). Direct observations of pelagic larval dispersal are almost impossible even with current technologies (Levin 2006). However, describing the genetic population structure for long distance dispersing species can provide an indirect measurement of larval dispersal, both in terms of distance and direction. The genetic data from multiple species can elucidate large scale patterns of marine connectivity, which may be used to identify areas and ecosystems for protection or to examine current protection boundaries (Kinlan & Gaines 2003; Gaines *et al.* 2003; Bradbury *et al.* 2008; Toonen 2011).

Benthic spawning marine invertebrates typically have a prolonged period of larval development during which they may be capable of dispersing great distances (Scheltema 1971; Kay & Palumbi 1978; Grantham *et al.* 2003). Similar to many bottom-dwelling marine organisms, sea urchins have a biphasic life cycle (Thorson 1950). As adults, sea urchins are bottom-dwelling and remain relatively sessile, but during mass spawning events they disperse their propagules into the water column. Tropical species develop for up to eight weeks, allowing larvae to be carried by currents and other oceanographic forces. Phenomena such as large permanent oceanic currents can potentially move larvae a great distance from their origin, but forces including local eddies can cause larvae to

settle close to their starting point (Lobel & Robinson 1986; Flament 1996, Paulay & Meyer 2002; Shanks 2003; Gaines 2003).

Sea urchins, and other mass spawning species, have the potential and ability to disperse their larvae great distances, and in some cases potential dispersal distances (larval duration) have been positively correlated to actual dispersal distance (Shanks *et al.* 2003) and geographic range size (Lester *et al.* 2007), but this is not the most common outcome (Weersing & Toonen 2009). It has become evident that, for many species, a significant number of larvae do not reach their full dispersal potential, instead settling near their home environment due to oceanographic factors, chemical cues, selection against immigrants, or barriers to migration (Barber *et al.* 2000; Swearer *et al.* 2002; Uthicke & Benzie 2003; Kinlan *et al.* 2005). Some benthic invertebrates that are able to disperse their larvae great distances may also have local larval retention resulting in subtle but significant genetic structure at smaller geographic scales, such as along a coastline or within island archipelagos (Sponaugle *et al.* 2002; Sotka *et al.* 2004; Bird *et al.* 2007; White *et al.* 2010; Skillings *et al.* 2011).

The Hawaiian archipelago, comprising 18 islands and atolls, is considered one of the world's most unique and pristine biological laboratories because of its geographic remoteness, large size, island hotspot formation, and unique ecological assemblage of native and endemic flora and fauna. Papahānaumokuākea Marine National Monument (PMNM), the largest marine protected area in the United States, also now a World Heritage Site, comprises the northwest region of the Hawaiian island chain and includes ten smaller relatively uninhabited islands and atolls. To the southeast lies the eight larger

main islands, of which the majority of coastline is not under protection due to occupation by human populations.

Three major prevailing currents are capable of transporting larvae of marine fauna along the archipelago (Flament 1996; Timmers *et al.* 2011). The Subtropical Countercurrent (SCC) and the Hawaiian Lee Countercurrent (HLC) flow from the northwestern Hawaiian islands (NWHI) towards the southeastern main Hawaiian islands (MHI) along the south of the Archipelago and the North Hawaiian Ridge Current (NHRC) flows from the MHI towards the NWHI along the north of the Archipelago (Flament, 1996; Timmers *et al.* 2011). One hope for PMNM is that the protected region will provide a refuge for populations of native fauna enabling them to flourish undisturbed. In addition to preserving local biodiversity, populations in the NWHIs can provide genetic stock and offspring to spill over into the adjacent largely unprotected MHIs and other nearby islands and atolls via these ocean current pathways. To test this hypothesis, it is necessary to determine if these regions exchange offspring, and if so, in what directions, and how far are the larvae able to travel before settling (Kinlan & Gaines 2003; Kinlan *et al.* 2005; Shanks *et al.* 2003). In addition to examining larval exchange within this archipelago, it is also important to determine if larvae are able to reach other nearby archipelagos. Kingman Reef, in the Line Islands, 1480 km to the southwest of Oahu is the second closest neighbor to Hawaiian Islands, and is known to have biologically similar marine flora and fauna (Skillings *et al.* 2011).

Sea urchins have been historically, and continue to be, one of the most commonly chosen groups of benthic invertebrates for studying genetic population structure of tropical and temperate marine biota due to their life history traits. Urchins have been the

focus of embryology and evolution research for more than a century, which led to the early availability of molecular markers for this group (Palumbi & Wilson 1990, Palumbi 1996a; Edmands *et al.* 1996; Debenham *et al.* 2000; Lessios *et al.* 2001, 2003; Flowers *et al.* 2002; Addison & Hart 2005).

Mass spawning events and long distance dispersal capabilities tend to result in low genetic differentiation at small spatial scales for benthic marine species such as sea urchins. However, at larger spatial scales, the variability tends to increase due to the stochastic nature of long distance dispersal, and the genetic pattern can vary greatly between species with similar dispersal capabilities (Johnson & Black 1982, 1984; Williams & Benzie 1997; Uthicke & Benzie 2003, Bird *et al.* 2007). Across the tropical Pacific region, long distance dispersing urchins, such as the pantropical *Tripneustes*, are genetically homogenous throughout the Indo-Pacific, while other urchin species, such as *Diadema*, show significant genetic structure within the same geographic range (Lessios *et al.* 2001, 2003). Other tropical Pacific benthic spawning echinoderms, such as the Indo-West Pacific sea stars *Linckia* and *Acanthaster*, show similar lack of genetic structure in the tens to hundreds of kilometer range but do show significant structure between widespread locations in the thousands of kilometer range (Williams & Benzie 1997; Yasuda *et al.* 2009; Timmers *et al.* 2011 ). In the central tropical Pacific, two more recent studies of the sea star, *Acanthaster*, and the sea cucumber, *Holothuria*, showed significant broad scale patterns of genetic structure between different island Archipelagos thousands of kilometers apart, but were also able to detect subtle genetic structure between some islands within the Hawaiian Archipelago within tens to hundreds of kilometers (Timmers *et al.* 2011; Skillings *et al.* 2011).

The use of multiple genetic markers to study marine population genetics has become more popular to increase the confidence in results, especially from multiple genomic regions (Gomez & Uchida 2003; Avise 2004; Burton 1996, 2009). Sequencing of the cytochrome oxidase subunit I (COI) gene is most one of the most common and widely applied molecular technique for examining marine population structure for tropical Pacific marine invertebrates (Palumbi 1996b, 1997; Benzie *et al.* 2002; Lessios *et al.* 2003; Bird *et al.* 2007; Duda & Lessios 2009; Skillings *et al.* 2011; Timmers *et al.* 2011). By contrast, amplified fragment length polymorphism (AFLP) markers provide a quick, reliable, and effective way of scanning the nuclear genome for large numbers of comparable loci relative to other nuclear methods such as restricted fragment length polymorphisms (RFLPs) or microsatellites (Vos *et al.* 1995; Baus *et al.* 2005; Bensch & Addison 2005; Garioa *et al.* 2007; Zhao *et al.* 2007; Yu & Chu 2006; Gomez-Uchida *et al.* 2003). For benthic mass spawning tropical marine animals, a nuclear marker should produce the same general conclusions as a mitochondrial marker because during periodic spawning events, vast amounts of urchin gametes freely mix with each other, which would not result in a difference in dispersal patterns between males and females as is seen with some vertebrates (Bowen *et al.* 2005).

Mitochondrial COI sequencing and genome wide AFLPs both have the capability to detect genetic structure at geographic scales from hundreds to thousands of kilometers. For species with long larval dispersal capabilities, using the standard of mitochondrial markers coupled with the use of a large number of nuclear markers may result in the detection of subtle genetic structure that would not have been resolved with a single genetic technique.

**Study species**

*Heterocentrotus mammillatus* (Linnaeus, 1758), the red pencil urchin, is a benthic echinoderm that is patchily distributed in the Indo-Pacific region (Figure 1). Individuals occur in large groups in areas of moderate to high wave energy and low sedimentation (Dotan 1990a, 1990b). The geographic range of the red pencil urchin spans from the Indian Ocean to the central Pacific Ocean, and the spotty distribution pattern, common in echinoderms due to the periodical mass spawning events, is evident in its central Pacific distribution. For example, the species appears absent from French Frigate Shoals, yet occurs on most other islands in the NWHIs (R. Toonen, pers. comm. 2005). Also, the species is abundant at Kingman Reef but is noticeably absent from its neighbor Palmyra Atoll which is only 53 km to the southeast (Y. Papastamatiou, pers. comm. 2005). The furthest eastern extent of the red pencil urchin distribution is the Hawaiian Archipelago.

The red pencil urchin is a broadcast spawner with robust larvae able to travel ocean currents in their pelagic form for weeks (Dotan 1990c). However, specific life history information about this species' life span, fertility, fecundity, and the pelagic larval duration (PLD) is lacking, so the response to oceanographic factors such as temperature and salinity and the distance traveled by the larvae remain a mystery. Studies of larval development (McEdward 1986a, 1986b) and reproductive information from populations in the Red Sea indicate that spawning occurs on a lunar or semi-lunar cycle and that the time from gametogenesis to maximum reproductive ripeness is about five months (Dotan 1990c).

Here I report on the genetic population structure of the red pencil urchin, *Heterocentrotus mammillatus*, from the most northeastern part of its range, the Hawaiian Archipelago and Kingman Reef. I used the mtDNA sequences of the cytochrome *c* oxidase subunit I (COI) gene and amplified fragment length polymorphism (AFLP) markers to: 1) to examine and describe how genetic variation of wild populations of the red pencil urchin is distributed throughout the Hawaiian Island chain, 2) to examine connectivity and genetic variability between the MHI and NWHI populations, and 3) to examine and describe genetic connectivity between the Hawaiian Archipelago and a nearby Line Islands Archipelago population at Kingman Reef.

## CHAPTER 2: MATERIALS AND METHODS

### Field sampling and laboratory procedures

A single urchin spine was collected nonlethanlly from each individual from seven sample populations of the red pencil urchin, *H. mammillatus* (Table 1, Figure 2). The thick spines of this urchin species, once used as chalk in old Hawai‘i (Titcomb 1978), had sufficient muscle tissue at the base (after removal) which was used for DNA extraction. Spine collections were made in water depths between 3–30 meters, which is typical of the species. Each spine was preserved in 95% ethanol in the field and held at ambient temperature (19-21ºC) for transport to the laboratory for DNA extraction. Total genomic DNA was extracted from approximately 25 mg of tissue from the base of each spine following the DNeasy Blood & Tissue extraction kit protocol (QIAGEN P/N 69504) and the DNA stock was divided in half for the two genetic analyses. The number of samples successfully processed for each method differed between the two (Table 1). A genetic reference sequence of the cytochrome oxidase subunit I gene (553bp COI sequence) was produced and deposited into GENBANK on September 12, 2007 (Accession # EU153189).

An approximately 410 bp region of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) was amplified using the primers GenHol2R (5-CTACCATTGCGTAGACCATTCC-3) and GenHol2L (5-GCATGAAAAACATGAGATTCTGAC-3), which had been modified from those used for the sea cucumber *Holothuria* spp. (Skillings *et al.* 2011). Reactions were performed using 20 μL volumes containing 0.4 μL of each primer, 2 μL of 1:49 diluted DNA and Biomix Red (Bioline Inc.) with an Icycler thermocycler (Bio-Rad Laboratories) using an

initial denaturation at 95° C for 1 minute followed by 35 cycles of denaturing at 94° C for 30 seconds, annealing at 51°C for 30 seconds and extension at 72° C for 45 seconds. A final extension at 72°C for 10 minutes preceded 4ºC refrigeration overnight. PCR products were cleaned by adding 7.5 units of *Exonuclease I* and 0.75 units of FastAP alkaline phosphatase (Fermentas Life Sciences) to the PCR products (7.5 μL) and incubating at 37°C for 60 minutes followed by an inactivation step at 85°C for 15 minutes. Sequencing was performed on an ABI 3130XL automatic sequencer. Samples were sequenced in the forward and reverse directions, and sequences were compiled, aligned and edited with SEQUENCHER version 4.8 and analyzed using ARLEQUIN version 3.5 (Excoffier & Lischer 2010).

Amplified fragment length polymorphism (AFLP) markers were identified using kits from Applied Biosystems (ABI). The restriction and ligation phases were carried out simultaneously by creating the enzyme master mix following the manufacturer recipe (with the adaptors) and adding 2.75 μL of the mix to 2.75 μL of DNA and leaving the mix overnight at ambient temperature. The completed reactions were diluted with water 1:1 prior to preselective amplification. A pre-selective amplification mix was created following manufacturer recipe (with primers) and 6 μL were added to 1.5 μL of the restriction/ligation mixture. Of twelve selective primer pair combinations examined for variability and repeatability, four were chosen for final analysis: *Eco*RI-ACT/*Mse*I-CTA, *Eco*RI-ACT/*Mse*I-CAG, *Eco*RI-ACT/*Mse*I-CTT, and *Eco*RI-ACT/*Mse*I-CTG. All PCR reactions were run on an MJ Research PTC-200 Peltier Thermal Cycler using a heated lid following the AFLP plant mapping protocol cycle recommendations from ABI. The selective amplification products were mixed with an internal size standard (ROX-500,

P/N 401098) and formamide for individual injection into an ABI Prism 3730*xl* DNA Analyzer. To avoid bias from different PCR plate runs, samples from multiple populations were put on the same plate and band scoring was done blindly with respect to the source population; additionally, multiple blank (water) samples were run simultaneously on plates with actual samples for quality control. To create blank samples, water was used in the place of DNA in the chemical reactions and the resulting peak heights were used to check for background noise and signal strength for non-DNA samples. For all other reactions, fragments were identified, sized and scored as either present or absent within each individual using ABI's GENEMAPPER 3.0.

**Data analysis**

Genetic diversity for the COI dataset was assessed by haplotype and nucleotide diversity. Haplotype diversity ($h$) was calculated by comparing the relative frequency of each unique haplotype to the total number of haplotypes within each population following: $h = (1 - \Sigma x_i^2)\, n / (n - 1)$ (Nei & Tajima 1981). Nucleotide diversity ($\pi$) was calculated by finding the average number of nucleotide differences between randomly selected sequences from each population (Nei & Li 1979). These diversity values were calculated using ARLEQUIN version 3.5 (Excoffier & Lischer 2010). I quantified genetic diversity for the AFLP data by the percentage of polymorphic loci ($PL$) and expected heterozygosity ($H_e$) generated with GENALEX version 6 (Peakall & Smouse 2006). For these calculations each fragment was treated as a single, unique gene locus with Mendelian segregation of a single dominant (amplified) and recessive (null) allele (Travis *et al.* 1996). To estimate $H_e$, I assumed Hardy-Weinberg proportions within populations.

Divergence among populations and island groups was assessed by an analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992; Excoffier & Smouse 1994). Paired genetic distances among the seven populations were estimated using $\Phi_{ST}$. The statistical significance of the variance components of the AMOVA and paired comparisons were determined from nonparametric procedures using 999 random permutations. Isolation by distance among the populations was assessed by Mantel tests using actual and log-transformed values of pairwise population geographic and genetic distances. The calculations were completed with the Isolation by Distance Web Service program version 3.16 (Jensen *et al.* 2005).

I also conducted analyses that were not dependent on *a priori*-defined populations. For the COI data, a haplotype network was constructed using NETWORK version 4.6 (Bandelt & Dress 1992). All individuals were color coded according to their population origin and the size of the node was proportional to the number of individuals with that haplotype. NETWORK PUBLISHER version 4.6 was used to produce and edit the haplotype network (Bandelt & Dress 1992). Lastly, a Bayesian analysis of the AFLP data with STRUCTURE 2.3.3 was used to assign individuals to putative genetic groups (Pritchard *et al.* 2000; Falush *et al.* 2003; Falush *et al.* 2007). An admixture ancestry model was assumed. Next, 5 replicates were run for $K$ from 1 – 8 using a burn-in period of 100,000 and a Markov chain Monte Carlo (MCMC) simulation of 100,000 iterations. The five replicates were run for both correlated and independent allele frequency models. Prior to running the simulations, the model parameter $\lambda$ was estimated using a single run of $K = 1$, as recommended by Pritchard *et al*. (2007). The number of genetic clusters in the dataset was determined by examining the posterior probabilities ($\ln \Pr(X|K)$ for the

varying values of *K*). In particular, a visual examination was used to identify when the change in posterior probabilities began to asymptote (Pritchard *et al*. 2000). After an appropriate *K* was identified, the individual assignment probabilities were examined.

# CHAPTER 3: RESULTS

## Genetic diversity

Forty-two haplotypes were identified among the 207 samples of *H. mammillatus* analyzed for COI variation (Table 1). Of these, 28 of were unique to a single sample. Of the 14 shared haplotypes, the average haplotype diversity for all seven populations was high overall at 0.85, and the range within populations was 0.80–0.89. The overall nucleotide diversity was low at 0.01, varying within sample populations from 0.005–0.008. The species level and population level estimates indicate that there are a relatively high number of haplotypes in each population, but the difference between any two sequences was low.

A total of 161 specimens of *H. mammillatus* were analyzed with AFLP markers, and 146 loci were identified. Among all populations, 121 (83%) loci were polymorphic (Table 2). Within populations, the percentage of polymorphic loci (*PL*) varied from 72% to 96%. Among all samples, the expected heterozygosity ($H_e$) was 0.418. The mean $H_e$ within populations was 0.358, with a range of 0.255–0.431.

## Divergence among populations

Among the six populations from Hawai‘i, only the AFLP dataset indicated there were significant differences among regions (main vs. northwestern islands) and populations within regions (Table 3). When examining the main versus the northwestern islands, both AMOVA analyses showed the majority of the variation within populations (99% for COI and 96% for AFLPs) and very little variation between regions; however, only the AFLP analysis identified statistically significant genetic structure. The COI AMOVA

resulted in insignificant regional values (-1.26%, p=1.00), failing to detect structure between the MHI populations and the NWHI populations. The AFLP AMOVA, however, found a small (1.2%) but significant ($p<0.05$) level of variation between groups.

Both the COI and AFLP datasets indicated significant differentiation between the populations from Hawai‘i and the Kingman Reef population (Table 4). When the six Hawaiian Archipelago sites were grouped together and compared to Kingman by itself, there was significant variation between regions (island chains) for both the COI and AFLP datasets. The vast majority of variation, 97% and 95% for the COI and AFLP data, respectively, was found within regions.

Pairwise population $\Phi_{ST}$ matrix for all seven populations showed mostly insignificant differentiation among populations for the COI data (Table 5). The population from Pihemanu was significantly different from the population at Holoikauaua as well as the population from Kingman Reef. No other paired comparisons indicated significant genetic structure. In contrast to the COI data set, most of the paired comparisons for the AFLP data indicated significant genetic structure, consistent with the AMOVA results. Kingman Reef was significantly differentiated from all six Hawaiian archipelago populations. The population from Kanemiloha‘i, the most northwestern location sampled, was also significantly differentiated from the remaining five populations sampled from the island chain. Pihemanu, the second furthest northwestern population sampled was not significantly differentiated from any other populations to the southeast in the chain. Holoikauaua of the NWHIs and O‘ahu from the MHIs were significantly differentiated from all populations except Pihemanu. The populations from

Kanemiloha‘i and Hawaii Island were not significantly different from each other or from Pihemanu.

There was scant evidence for isolation-by-distance among populations, either among the populations within the Hawaiian archipelago alone or including the population at Kingman Reef (Table 6). Only the comparison of the log (geographic) vs. genetic distance calculated for the AFLP markers for the Hawaiian Archipelago populations (excluding Kingman Reef) showed a significant IBD relationship ($r^2 = 0.322$, $P = 0.005$).

The haplotype network showed no obvious correspondence between haplotype and geographic location (Figure 3). Haplotypes were not clustered into distinguishable groups and revealed no clear geographic pattern. Out of the total 42 haplotypes, three of four most common haplotypes were found in all seven populations. Although the Line Island population at Kingman Reef did have the smallest number of shared haplotypes and largest number of singleton haplotypes, it did not present itself as differentiated from the rest of the populations.

The structure analysis indicated there were two clusters in the AFLP data set (Figure 4). However, there was no geographic pattern present (Figure 5); a single common cluster was found in all populations and a second cluster was found primarily in Hawai‘i Island, Holoikauaua, Pihemanu, and Kingman Reef.

## CHAPTER 4: DISCUSSION

The red pencil urchin, *Heterocentrotus mammillatus*, is a genetically diverse marine species but individuals are closely related to each other, suggesting ongoing but sporadic gene flow throughout the entire study range in the central North Pacific. Mitochondrial COI haplotype diversity was high for all seven populations, although nucleotide diversity was low. AFLP expected heterozygosity was high for all populations, and each individual had a unique AFLP banding pattern as seen in other AFLP analyses of tropical wild marine invertebrate populations (Baus *et al.* 2005), indicating a significant level of genetic variability within each population.

The patterns detected with the mtDNA marker COI and nuclear AFLP markers were largely concordant indicating that the majority of the genetic variability lies between individuals within populations, which is common for marine species (Uthicke 2003; Bird *et al.* 2007). Interestingly, when examining genetic structure throughout the entire study range, there was a lack of significant genetic structure using COI, while the AFLP data did detect low but significant levels of structure. No geographic pattern of genetic variation was evident using either method. These data suggest ongoing gene flow between all Hawaiian populations and Kingman Reef, but not enough gene flow to create one homogenous population throughout the North Pacific sample range. Over 90% of the variation was found between individuals within populations and within regions; only a small portion of the total variation was found among regions, indicating a lack of substantial dispersal barriers for this species.

Both the COI and AFLP data indicated low but significant levels of differentiation between the two island archipelagos (Hawaii and Kingman Reef as a representative of the Line Islands). This indicates limited larval exchange despite some level of ongoing gene flow as evidence by the shared haplotypes (Table 2). Because there were unique haplotypes in each area, the barrier is likely to be bi-directional. For example, the Kingman Reef population had the highest number of unique COI haplotypes and the lowest number of shared haplotypes out of all other populations in this study. The AMOVA analysis between Kingman and Hawaii detected low but significant variation between the archipelagos for both the COI and AFLP data. The pairwise $\Phi_{ST}$ comparisons for AFLP data only showed Kingman significantly differed from all other populations, also indicative of barriers to gene flow between the island groups. Following these trends, the data for both genetic methods did not fit any isolation by distance (IBD) model along the Hawaiian Archipelago, except one analysis of the AFLP data. When all seven populations were analyzed together both IBD results remained insignificant.

Given these patterns, it was not surprising that the visual methods of interpreting the genetic data for each genetic technique were unable to produce a clear geographic pattern of genetic variation. The COI haplotype network showed that each of the most common haplotypes were shared throughout the entire study range. Although Kingman Reef was significantly different from the Hawaiian populations in the AMOVA analysis, the haplotype network did not reveal the single representative of the Line Island Archipelago as a geographic outgroup or being substantially differentiated from the rest of the Hawaiian Archipelago. However, this could change if additional populations from the Line Islands were included, such as Jarvis Island.

This unusually high level of connectivity at such a large geographic scale (1500 km) was also found in the study of the sea cucumber *Holothuria atra* (Skillings *et al.* 2010). Thus, Kingman Reef may be a stepping stone between Hawai‘i and the rest of the Pacific — a center of genetic diversity rather than a dead end with regard to echinoderms. A visual survey for the red pencil urchin was conducted on Palmyra Atoll, approximately 50 km away from Kingman Reef. While the beach was littered with dead pencil urchin spines, no live animals reported from the reefs (C. Zabin, pers. comm. 2006) which is somewhat analogous to the highly limited gene flow between the two adjacent locations detected by Skillings *et al.* (2010) for *H. atra*. Interestingly, for the red pencil urchin, despite showing connectivity across 1500 km to Hawaii, there was none across 50 km to Palmyra.

In the tropical Pacific, broadcast spawning sea urchins and other benthic spawning invertebrates have long dispersal capabilities due to robust larvae that can spend weeks in the water column travelling long distance via oceanographic forces such as currents and eddies (Palumbi & Wilson1990; Grosberg 2001). Populations of spawning species tend to be well mixed at local and regional levels of tens to hundreds of kilometers, but can show broad scale structure over thousands of kilometers. For example, this pattern of local homogeneity with broad-scale structure has been detected in organisms as varied as the tropical Pacific cone snail, *Conus ebraeus* (Duda & Lessios 2009), the sea urchin, *Echinometra* (Palumbi 1996a, 1997), and the coconut crab, *Birgus latro* (Lavery *et al.* 1996). It is important to remember that the total distance a larvae travels before settling does not equate directly to geographic distance (White *et al.* 2010; Selkoe *et al.* 2010), so while many larvae are able to travel great distances through

currents, some larvae can get caught by local oceanographic forces and stay close to their starting point, creating low levels of structure between geographically close locations (White *et al.* 2010; Selkoe *et al.* 2010). The use of genetic nuclear markers such as anonymous nuclear loci (anDNA) has helped to identify these low levels of genetic structure at smaller spatial scales (Arnaud-Haond *et al.* 2003) that may otherwise remain undetected with the use of mitochondrial markers such as COI.

Within the Hawaiian Archipelago, there was little statistical support for significant genetic structure, although differences between the marker datasets was seen. Pairwise comparisons identified only two significant values for the COI data, yet most pairwise comparisons made with the AFLP dataset indicated significant genetic structure. Neither data set showed any evidence for isolation-by-distance nor did any geographic pattern to the genetic variation emerge, suggesting stochastic dispersal throughout the entire island chain.

A strange pattern has been emerging in literature between the island of Hawaii and some of the northwestern Hawaiian Islands with other invertebrates that is not explained by current oceanographic models of established currents and local eddies. The COI dataset of this study indicates strong connectivity between the west side of Hawai‘i island to the rest of the archipelago, which contrasts with Timmers *et al.* (2011) recent analysis of the crown-of-thorns sea star, *Acanthanster planci*, that found the majority of their other Hawaiian archipelago sites significantly different from the west Hawaii island site. These patterns are possibly due to local recruitment driven by surface currents produced by the trade winds (Flament, 1996). However, the AFLP data did identify west Hawai‘i as significantly different from all other sites except for two of the populations

from the northwestern Hawaiian islands: Holoikauaua and Pihemanu. The latter population was the only sample site in this study that was not significantly different from the remainder of the populations. The results of both genetic analyses show connectivity between Hawaii Island and parts of the NWHIs, but the AFLP analysis was able to distinguish subtle differences throughout the remainder of the Hawaiian Archipelago that was undetected in the COI analysis.

A genetic split between the main and northwestern Hawaiian Islands is present in some marine invertebrate species, but not in all (Toonen *et al.* 2011). In three separate population genetic COI studies of benthic marine spawning invertebrates in the Hawaiian Islands, the studies of the mollusk *Cellana* (Bird *et al.* 2007) and the sea cucumber *Holothuria* (Skillings *et al.* 2010) did show low but significant variation between the two regions, but the study of the coral eating starfish *Acanthaster* did not (Timmers *et al.* 2011). For *H. mammillatus*, only the AFLP data were able to detect significant variation between the main and northwestern portions of the chain, although both datasets indicated similar amounts of genetic structure. These genetic diversity results were comparable to those of the crown-of-thorns sea star, which is found in similar habitat types and depth ranges as the red pencil urchin. Furthermore, there was also a lack of COI variation between the two island groups for the sea star. Had it not been for the AFLP data set, the weak but significant genetic structure between the regions would not have been discovered for this urchin species. This suggests that other population genetic studies of benthic marine species may benefit from the same type of additional nuclear markers.

While gene flow in *Cellana* (Bird *et al.* 2007) and *Holothuria* (Skillings *et al.* 2010) indicated a substantially higher migration rate from the MHIs to the NWHIs, in the more ecologically similar species, *Acanthaster* (Timmers *et al.* 2011), gene flow did not appear to be quite as unidirectional. These findings suggest that the larvae of the red pencil urchin may be dispersing, similar to *Acanthaster* in both directions along the Hawaiian Archipelago.

The red pencil urchin appears to have long-distance dispersal capabilities, across 1500 km of ocean to Kingman Reef and from one end of the Hawaiian Archipelago to the other 2500 km away. Both genetic techniques told the same story of low variation throughout the island chain and low variation between the Hawaiian populations and Kingman Reef. However, AFLP markers detected more significant levels of variation between populations, regions, and island chains. *Heterocentrotus mammillatus* is a benthic spawning species that shows throughout Hawaii, there is high connectedness between the protected northwestern and largely unprotected main islands as well as between the northwestern Hawaiian Islands and Kingman Reef. These data suggest that larvae are successfully transported from the NWHIs to the surrounding areas but that the long distance dispersal events are most likely rare.

**Conclusions**

Using multiple genetic techniques I was able to identify a more complete picture of the genetic structure of the red pencil urchin throughout a large part of its natural range. Although the results of both markers were largely comparable, I was able to detect slightly more resolution and significance with the nuclear markers. This study thus

highlights the advantages of using multiple markers and provides a case study to highlight the usefulness of AFLP markers for population genetic studies over thousands of kilometers.

This study illustrates that the red pencil urchin is able to successfully disperse its larvae from one end of the Hawaiian Island Chain to the other (over 2500 km) and to Kingman Reef (ca. 1500 km away). There was scant evidence of oceanographic barriers to stop the dispersal, including channel cross currents, wake eddies, and total distance between populations, but some barriers must exist due to the absence of this species at French Frigate Shoals (within the NWHIs) and at Palmyra (neighbor to Kingman Reef). In addition to minimal dispersal limitations, the stepping stone arrangement of the Hawaiian island chain and the three major currents that move along the chain in both directions most likely make for easy larval transport for this species and contribute to the connectivity of this species throughout the island chain. While other tropical Pacific echinoderms tell similar stories of high connectivity throughout the Hawaiian Archipelago with a few distinct differences, the accumulation of genetic information for multiple species and groups enables shared genetic breaks and areas of high connectivity to be resolved.

The results of this study will be added to the pool of current research attempting to outline shared genetic breaks throughout the Hawaiian Archipelago using population structure studies of wild native fauna. This will be useful for examining the effectiveness of the Papahānaumokuākea Marine National Monument and to help in proper management of the protected region (Toonen *et al.* 2011). For highly dispersing sessile invertebrate species that are subtidal, occur in large numbers, and have a relatively long

pelagic larval duration, AFLP analyses may provide additional resolution to existing COI data where there is a lack of significant genetic structure.

What does this study reveal in terms of the effectiveness of the Papahānaumokuākea Marine National Monument as a marine protected area? For as the red pencil urchin, *H. mammillatus*, these data suggest there is bi-directional migration of propagules between the mainly unprotected main islands and the now strictly protected northwestern islands regions. Thus, the northwestern Hawaiian Islands do appear to supply at least some larvae to the main islands and nearby archipelagos, and so Papahānaumokuākea seems to be effective in providing genetic stock to surrounding areas for some species, although definitely not all (Bird *et al.* 2007; Skillings *et al.* 2010; Toonen *et al.* 2011).

Based on the multiple benthic invertebrates studied throughout the Hawaiian Archipelago and the surrounding tropical Pacific islands and atolls, local oceanographic phenomenon appear to be very important in the transportation and dispersal of pelagic larvae, but each species is affected differently by those forces, resulting in unique geographic and genetic distribution patterns (Bird *et al.* 2007; Timmers *et al.* 2010; Skillings *et al.* 2010). While geographic range size, habitat depth, pelagic larval distance, and oceanographic forces alone are not sufficient to explain distribution patterns of marine fauna, multiple factors together with results of previous studies are able to produce patterns of genetic connectivity throughout a marine system such as the tropical Indo-Pacific. This study was able to highlight the importance of multiple genetic techniques to resolve subtle population structure of a previously undescribed marine species, and the results will add to existing genetic population data for other species

throughout this region which will be used to examine the effectiveness of the United States' largest marine protected area.

**Table 1** Population information for the seven sample locations in this study including previous (but still valid) name, current Hawaiian name, and number of samples from each population successfully processed and analyzed for both genetic techniques (*N*).

| Location (Old Names) | Location (Current Names) | *N* COI | *N* AFLP |
|---|---|---|---|
| 1 Hawai‘i | Hawai‘i | 28 | 22 |
| 2 O‘ahu | O‘ahu | 30 | 22 |
| 3 Kaua‘i | Kaua‘i | 28 | 21 |
| 4 Pearl & Hermes | Holoikauaua | 29 | 27 |
| 5 Midway Atoll | Pihemanu | 31 | 24 |
| 6 Kure Atoll | Kanemiloha‘i | 30 | 15 |
| 7 Kingman | Kingman | 31 | 30 |
| | Total | 207 | 161 |

**Table 2** Descriptive haplotype genetic diversity statistics for *Heterocentrotus mammillatus* along the Hawaiian Archipelago and Kingman Reef.

| Location | No. of haplotypes | Shared haplotypes | Unique haplotypes | Haplotype diversity | Nucleotide diversity |
|---|---|---|---|---|---|
| 1 Hawai‘i | 11 | 9 | 2 | 0.878 | 0.008 |
| 2 O‘ahu | 12 | 10 | 2 | 0.890 | 0.007 |
| 3 Kaua‘i | 15 | 8 | 6 | 0.884 | 0.008 |
| 4 Holoikauaua | 11 | 8 | 3 | 0.837 | 0.005 |
| 5 Pihemanu | 12 | 9 | 3 | 0.847 | 0.009 |
| 6 Kanemiloha‘i | 12 | 7 | 5 | 0.798 | 0.007 |
| 7 Kingman | 14 | 7 | 7 | 0.839 | 0.006 |
| Total | | | 28 | | |

**Table 3** AFLP genetic diversity statistics for *Heterocentrotus mammillatus* along the Hawaiian Archipelago and Kingman Reef including percent polymorphic loci (*PL*) and expected heterozygosity ($H_e$) for each sample population.

| Location | *PL* | $H_e$ |
|---|---|---|
| 1 Hawai‘i | 91.8 | 0.401 |
| 2 O‘ahu | 58.9 | 0.256 |
| 3 Kaua‘i | 71.9 | 0.301 |
| 4 Holoikauaua | 89.7 | 0.395 |
| 5 Pihemanu | 95.9 | 0.428 |
| 6 Kanemiloha‘i | 92.5 | 0.399 |
| 7 Kingman | 80.1 | 0.312 |
| Species | 98.63 | 0.418 |

**Table 4** Analysis of molecular variance results for *Heterocentrotus mammillatus* in the Hawaiian islands, subdivided by regions.

| Source of variation | *df* | Sum of squares | Variance component | % Total Variation | *p*-value |
|---|---|---|---|---|---|
| **COI data** | | | | | |
| Between regions | 1 | 0.68 | -0.02 | -1.26 | 1.000 |
| Among populations within regions | 4 | 9.49 | 0.03 | 1.92 | 0.095 |
| Within populations | 170 | 257.47 | 1.51 | 99.35 | |
| **AFLP data** | | | | | |
| Between regions | 1 | 51.02 | 0.26 | 1.2 | 0.012 |
| Among populations within regions | 4 | 134.84 | 0.63 | 3.0 | 0.001 |
| Within populations | 125 | 2523.28 | 20.19 | 95.8 | |

**Table 5** Analysis of molecular variance between populations of *Heterocentrotus mammillatus* sampled from the Hawaiian islands and Kingman Reef.

| Source of variation | *df* | Sum of squares | Variance component | % Total Variation | *p*-value |
|---|---|---|---|---|---|
| **COI data** | | | | | |
| Between regions | 1 | 4.61 | 0.05 | 3.11 | 0.031 |
| Within regions | 205 | 307.19 | 1.50 | 96.89 | |
| **AFLP data** | | | | | |
| Among regions | 1 | 71.61 | 1.06 | 5.1 | 0.001 |
| Within regions | 159 | 3142.71 | 19.76 | 94.9 | |

**Table 6** Pairwise differentiation ($\Phi_{ST}$) among seven populations of *Heterocentrotus mammillatus*. COI values are in the upper triangle, and AFLP values are in the lower triangle. Probability values were calculated from 999 permutations, and Bonferroni-corrected significant ($P \leq 0.00238$) values are shown in bold. Noncorrected levels of significance are indicated with asterisks.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1 Hawai‘i | – | -0.008 | -0.017 | 0.016 | 0.019 | -0.008 | 0.035 |
| 2 O‘ahu | 0.026* | – | -0.009 | -0.019 | 0.048 | -0.021 | 0.011 |
| 3 Kaua‘i | 0.033** | 0.029** | – | 0.011 | 0.011 | -0.020 | 0.042 |
| 4 Holoikauaua | 0.017 | 0.024** | 0.025* | – | 0.102* | -0.017 | -0.004 |
| 5 Pihemanu | 0.006 | 0.025* | 0.033* | 0.004 | – | 0.044 | **0.139*** ** |
| 6 Kanemiloha‘i | 0.063** | **0.132*** ** | **0.125*** ** | 0.068** | 0.043** | – | 0.017 |
| 7 Kingman | **0.073*** ** | **0.065*** ** | **0.086*** ** | **0.066*** ** | **0.072*** ** | **0.145*** ** | – |

*$P \leq 0.05$

**$P \leq 0.01$

***$P \leq 0.001$

**Table 7** Test of isolation by distance among the seven populations of *Heterocentrotus mammillatus*. Significant relationships are indicated in bold.

| | Hawaiian Islands | Hawai‘i + Kingman Reef |
|---|---|---|
| **COI data** | | |
| Geographic vs. Genetic | $r^2 = 0.098$ | $r^2 = 0.000$ |
| Log (Geographic) vs. Genetic | $r^2 = 0.143$ | $r^2 = 0.005$ |
| Geographic vs. Log(Genetic) | $r^2 = 0.006$ | $r^2 = 0.005$ |
| Log (Geographic) vs. Log(Genetic) | $r^2 = 0.013$ | $r^2 = 0.002$ |
| **AFLP data** | | |
| Geographic vs. Genetic | $r^2 = 0.000$ | $r^2 = 0.139$ |
| Log (Geographic) vs. Genetic | **$r^2 = 0.322$** | $r^2 = 0.004$ |
| Geographic vs. Log(Genetic) | $r^2$ = N/A | $r^2 = 0.010$ |
| Log (Geographic) vs. Log(Genetic) | $r^2$ = N/A | $r^2 = 0.023$ |

**Figure 1** Geographic distribution of *Heterocentrotus mammillatus* (Linnaeus, 1758). Distribution information includes historical references, Bishop Museum collection database information, online global biodiversity databases, and personal observations. Modified map produced using free online Global Biodiversity Information Facility (GBIF) software and Photoshop for final editing. *(Accessed through GBIF Data Portal, data.gbif.org, 2007-01-05)*

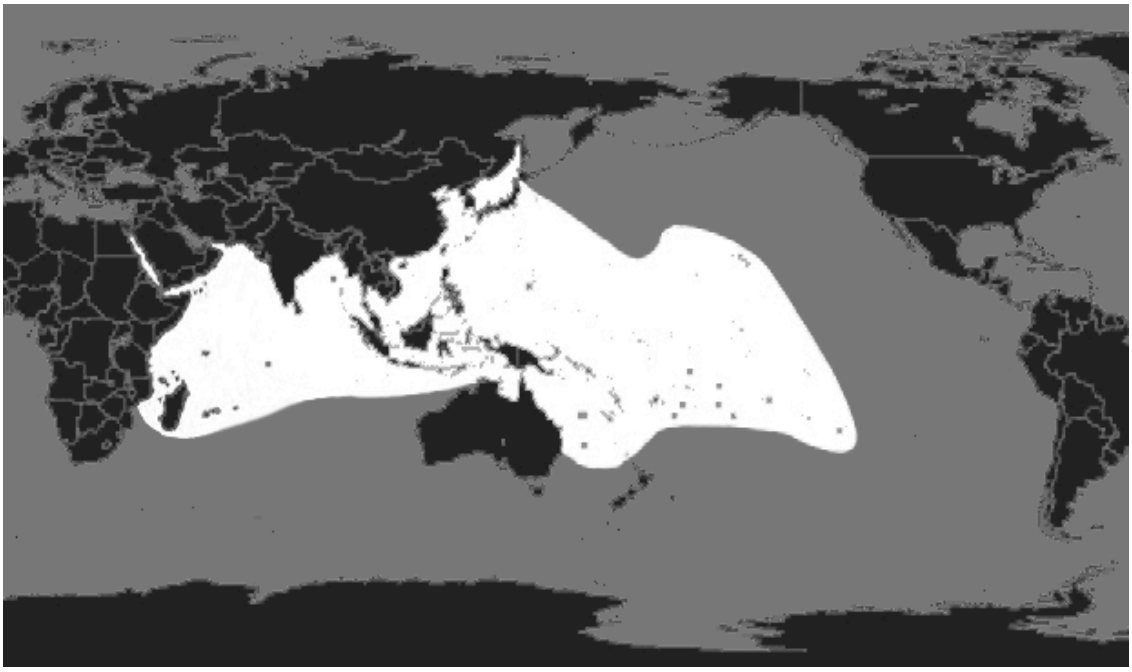


**Figure 2** Map of the central North Pacific Ocean indicating the seven sampling locations. The three major currents around the Hawaiian archipelago are designated by labeled arrows: the North Hawaiian Ridge Current (NHRC), Subtropical Counter Current (SCC), and Hawaiian Lee Counter Current (HLCC).

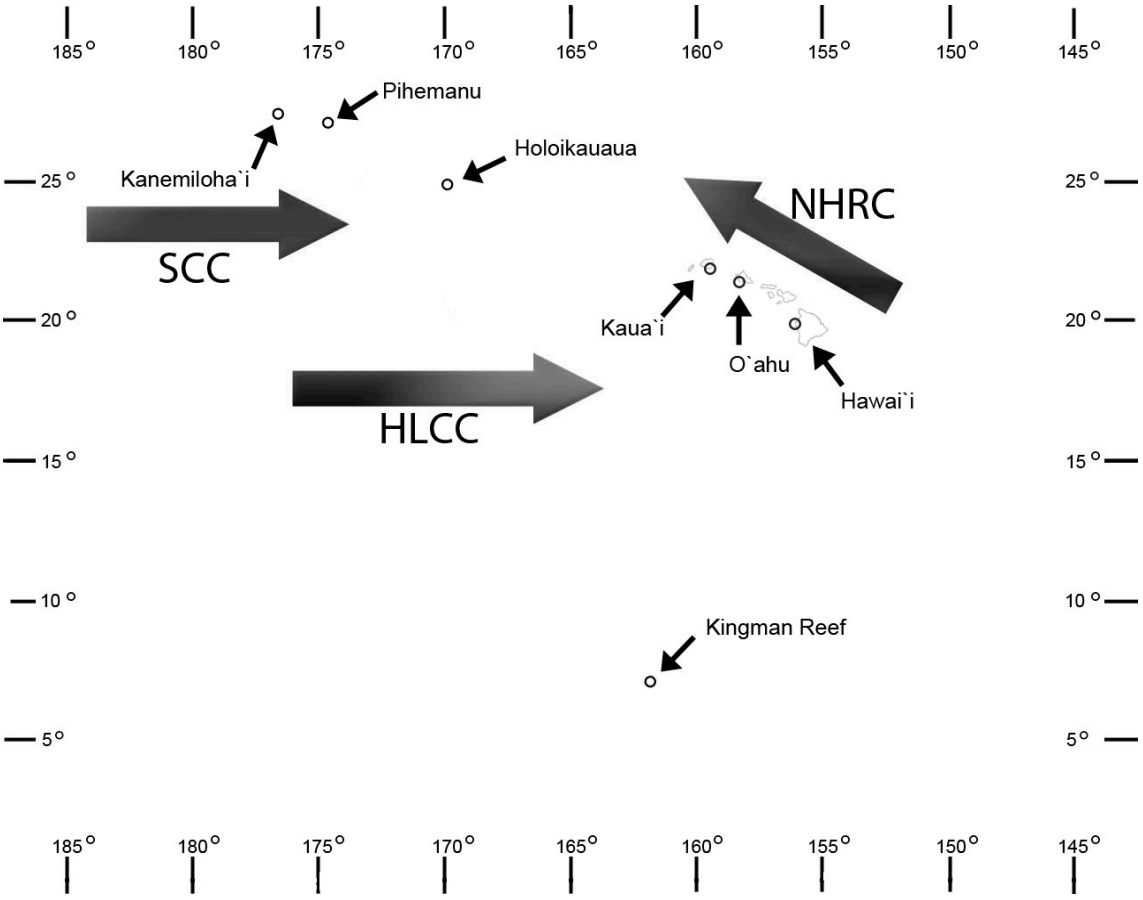


**Figure 3** COI median joining haplotype network of *Heterocentrotus mammillatus* sampled from the Hawaiian islands and Kingman Reef. Population origins are indicated by color on the map and network. Each circle represents a unique haplotype, and lines between haplotypes represent one or more base pair substitutions. For shared haplotypes, the size of the pie piece within each circle is proportional to its frequency.

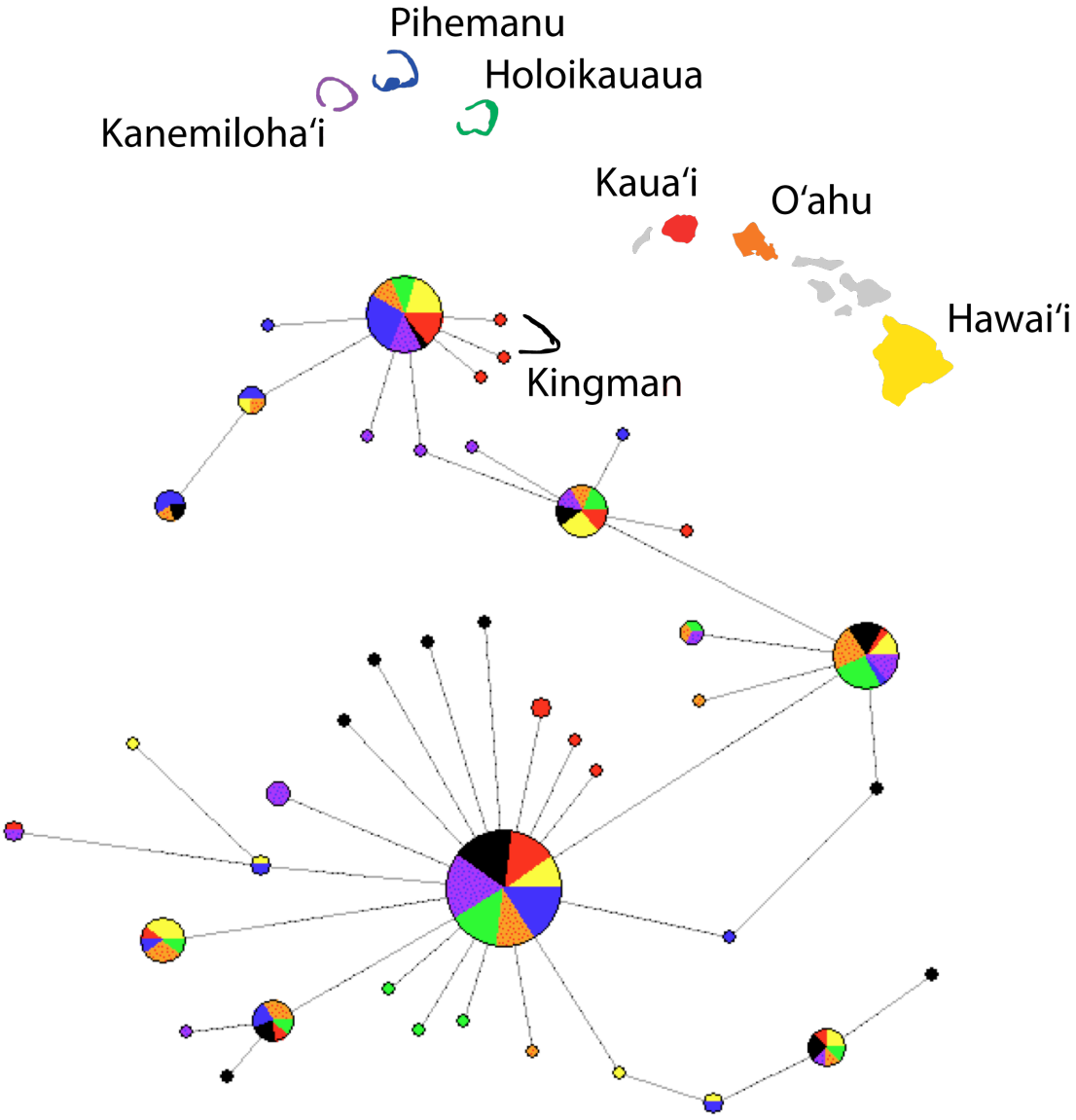


**Figure 4** Likelihoods for the number of *structure*-defined genetic clusters detected among the 161 individuals of *Heterocentrotus mammilatus* analyzed for AFLP diversity. For each of the two allele frequency models (Correlated = open circles; Independent = cross), we analyzed five simulations with a burnin of 100,000 and 100,000 MCMC replicates.

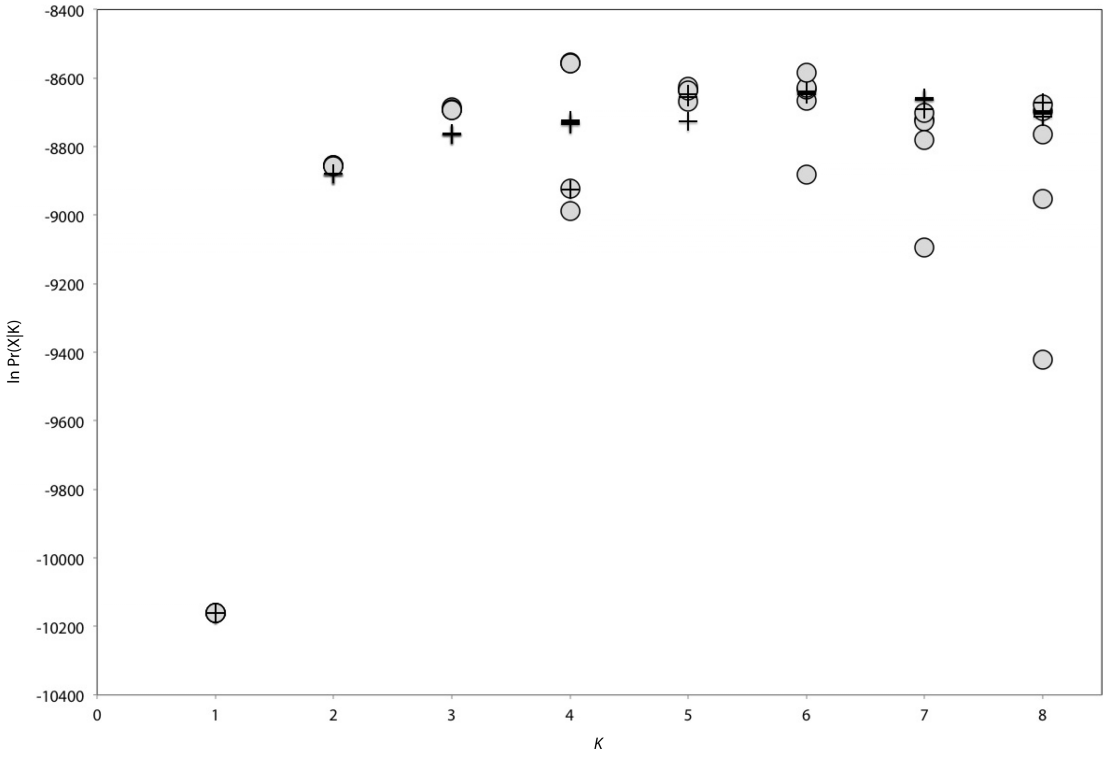
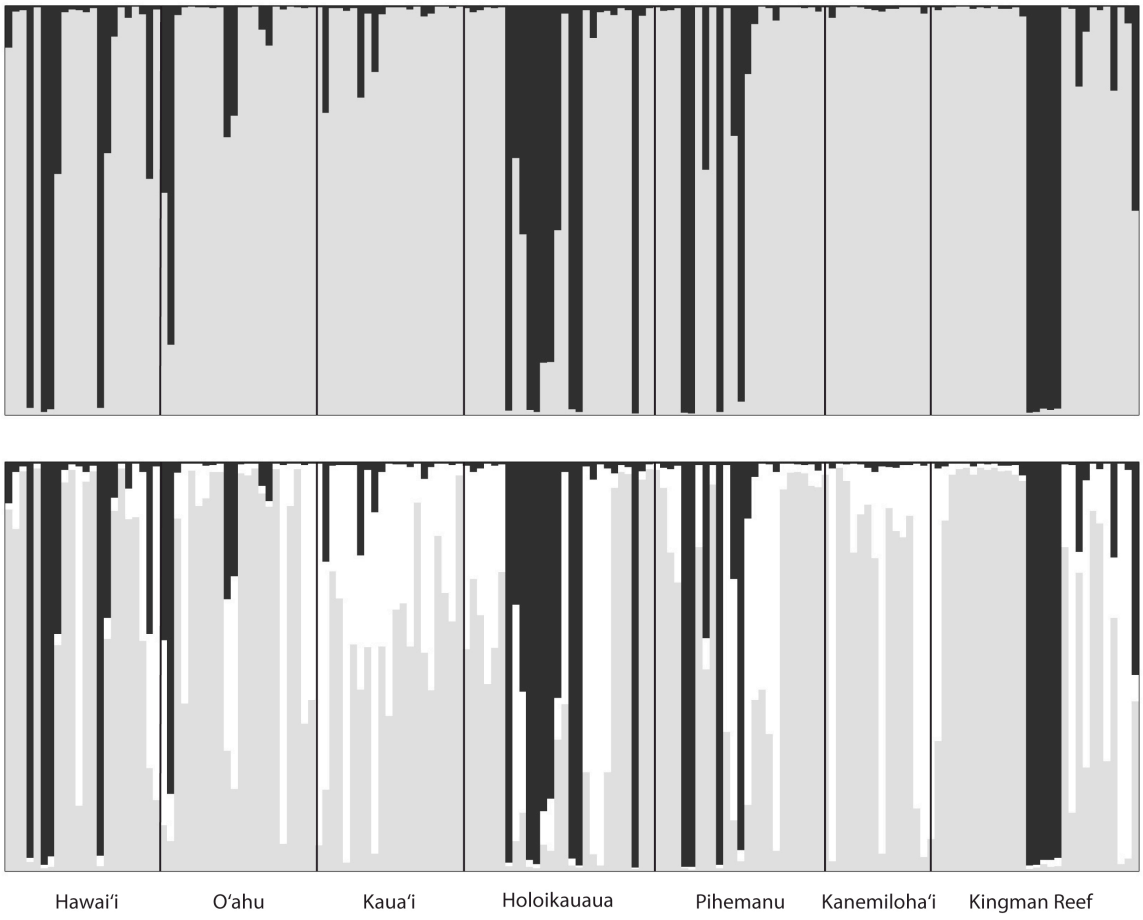


**Figure 5** Structure results for $K = 2$ and 3 subpopulations for AFLP data of *Heterocentrotus mammillatus*. Each vertical line corresponds to an individual sample, with the shading indicating the assignment probabilities to each of the hypothesized clusters.



## REFERENCES

Addison, J.A. and Hart, M.W. (2005) Colonization, dispersal, and hybridization influence phylogeography of North Atlantic sea urchins (*Strongylochntrotus droebachiensis*). *Evolution*, **59,** 532-543.

Arnaud-Haond, S., Bonhomme, F. and Blanc, F. (2003) Large discrepancies in differentiation of allozymes, nuclear and mitochondrial DNA loci in recently founded Pacific populations of the pearl oyster Pinctada margaritifera. *Journal of Evolutionary Biology*, **16,** 388-398.

Avise, J.C. (2004) *Molecular Markers, Natural History, and Evolution*, 2nd Edition. Sinauer, Sunderland, MA.

Bandelt, H. and Dress, A.W.M. (1992) Split decomposition: A new and useful approach to phylogenetic analysis of distance data. *Molecular Phylogenetics and Evolution*, **1,** 242-252.

Barber, P.H., Palumbi, S.R., Erdmann, M.V. and Moosa, M.K. (2000) Biogeography: A marine Wallace's line? *Nature*, **406,** 692-693.

Baums, I.B. and Paris, C.B. (2006) A bio-oceanographic filter to larval dispersal in a reef-building coral. *Limnology and Oceanography*, **51,** 1969-1981.

Baus, E., Darrock, D.J. and Bruford, M.W. (2005) Gene-flow patterns in Atlantic and Mediterranean populations of the Lusitanian sea star *Asterina gibbosa*. *Molecular Ecology*, **14,** 3373-3382.

Bensch, S. and Akesson, M. (2005) Ten years of AFLP in ecology and evolution: why so few animals? *Molecular Ecology*, **14,** 2899-2914.

Benzie, J.A.H., Ballment, E., Forbes, A.T., Demetriades, N.T., Sugama, K., Haryanti and Moria, S. (2002) Mitochondrial DNA variation in Indo-Pacific populations of the giant tiger prawn, *Penaeus monodon*. *Molecular Ecology*, **11,** 2553-2569.

Bird, C.E., Holland, B.S., Bowen, B.W. and Toonen, R.J. (2007) Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories. *Molecular Ecology*, **16,** 3173-3186.

Bowen, B.W., Bass, A.L., Soares, L. and Toonen, R.J. (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). *Molecular Ecology*, **14,** 2389-2402.

Bradbury, I.R., Laurel, B., Snelgrove, P.V.R., Bentzen, P. and Campana, S.E. (2008) Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. *Proceedings of the Royal Society B*, **275,** 1803-1809.

Burton, R.S. (1996) Molecular tools in marine ecology. *Journal of Experimental Marine Biology and Ecology*, **200,** 85-101.

Burton, R.S. (2009) Molecular Markers, Natural History, and Conservation of Marine Animals. *Bioscience*, **59,** 831-840.

Debenham, P., Brzezinski, M., Foltz, K. and Gaines, S. (2000) Genetic structure of populations of the red sea urchin, *Strongylocentrotus franciscanus*. *Journal of*

*Experimental Marine Biology and Ecology*, **253,** 49-62.

Dotan, A. (1990a) Population structure of the echinoid *Heterocentrotus mammillatus* L. along the littoral zone of southeastern Sinai Egypt. *Coral Reefs*, **9,** 75-80.

Dotan, A. (1990b) Distribution of regular sea urchins on coral reefs near the south-eastern tip of the Sinai Peninsula Red Sea. *Israel Journal of Zoology*, **37,** 15-29.

Dotan, A. (1990c) Reproduction of the slate pencil sea urchin *Heterocentrotus mammillatus* L. in the northern Red Sea. *Australian Journal of Marine and Freshwater Research*, **41,** 457-466.

Duda, T.F. and Lessios, H.A. (2009) Connectivity of populations within and between major biogeographic regions of the tropical Pacific in *Conus ebraeus*, a widespread marine gastropod. *Coral Reefs*, **28,** 651-659.

Edmands, S., Moberg, P.E. and Burton, R.S. (1996) Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*. *Marine Biology*, **126,** 443-450.

Excoffier, L. and Lischer, H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10,** 564-567.

Excoffier, L. and Smouse, P.E. (1994) Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: molecular vairance parsimony. *Genetics*, **136,** 343-359.

Excoffier, L., Smouse, P.E. and Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131,** 479-491.

Falush, D., Stephens, M. and Pritchard, J.K. (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164,** 1567-1587.

Falush, D., Stephens, M. and Pritchard, J.K. (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, 1-5.

Flament, P., Kennan, S., Lumpkin, M., Sawyer, M., and Stroup, E.D. (1996) *The Ocean Atlas of Hawaii*, University of Hawaii Press, Honolulu, Hawaii.

Flowers, J.M., Schroeter, S.C. and Burton, R.S. (2002) The recruitment sweepstakes has many winners: Genetic evidence from the sea urchin *Strongylocentrotus purpuratus*. *Evolution*, **56,** 1445-1453.

Fogarty, M.J. and Botsford, L.W. (2007) Population connectivity and spatial management of marine fisheries. *Oceanography*, **20,** 112-123.

Gaines, S.D., Gaylord, B. and Largier, J.L. (2003) Avoiding current oversights in marine reserve design. *Ecological Applications*, **13,** 32-46.

Galindo, H.M., Olson, D.B. and Palumbi, S.R. (2006) Seascape genetics: A coupled oceanographic-genetic model predicts population structure of Caribbean corals. *Current*

*Biology : CB*, **16,** 1622-1626.

Garoia, F., Guarniero, I., Grifoni, D., Marzola, S. and Tinti, F. (2007) Comparative analysis of AFLPs and SSRs efficiency in resolving population genetic structure of Mediterranean *Solea vulgaris*. *Molecular Ecology*, **16,** 1377-1387.

Gomez-Uchida, D., Weetman, D., Hauser, L., Galleguillos, R. and Retamal, M. (2003) Allozyme and AFLP analyses of genetic population structure in the hairy edible crab *Cancer setosus* from the Chilean coast. *Journal of Crustacean Biology*, **23,** 486-494.

Grantham, B.A., Eckert, G.L. and Shanks, A.L. (2003) Dispersal potential of marine invertebrates in diverse habitats. *Ecological Applications*, **13,** 108-116.

Grosberg, R. and Cunningham, C.W. (2001) "Genetic structure in the sea: from populations to communites," in *Marine Community Ecology*, M. D. Bertness, S. D. Gaines, and M. E. Hay, Eds., pp. 61–84, Sinauer Associates, Sunderland, Massachutes.

Jensen, J.L., Bohonak, A.J. and Kelley, S.T. (2005) Isolation by distance, web service. *BMC Genetics*, **6,** 1-6.

Johnson, M.S. and Black, R. (1982) Chaotic genetic patchiness in an intertidal limpet, <i>Siphonaria</i> sp. *Marine Biology*, **70,** 157-164.

Johnson, M.S. and Black, R. (1984) Pattern beneath the chaos: The effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution*, **38,** 1371-1383.

Kinlan, B.P. and Gaines, S.D. (2003) Propagule dispersal in marine and terrestrial

environments: A community perspective. *Ecology*, **84,** 2007-2020.

Kinlan, B.P., Gaines, S.D. and Lester, S.E. (2005) Propagule dispersal and the scales of marine community process. *Diversity and Distributions*, **11,** 139-148.

Lavery, S., Moritz, C. and Fielder, D.R. (1996) Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro*. *Molecular Ecology*, **5,** 557-570.

Lessios, H.A., Kane, J., Robertson, D.R. and Wallis, G. (2003) Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. *Evolution*, **57,** 2026-2036.

Lessios, H.A., Kessing, B.D. and Pearse, J.S. (2001) Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. *Evolution*, **55,** 955-975.

Lessios, H.A., Kessing, B.D. and Robertson, D.R. (1998) Massive gene flow across the world's most potent marine biogeographic barrier. *Proceedings: Biological Sciences*, **265,** 583-588.

Lester, S.E., Ruttenberg, B.I., Gaines, S.D. and Kinlan, B.P. (2007) The relationship between dispersal ability and geographic range size. *Ecology Letters*, **10,** 745-758.

Levin, L.A. (2006) Recent progress in understanding larval dispersal: new directions and digressions. *Integrative and Comparative Biology*, **46,** 282-297.

Lobel, P.S. and Robinson, A.R. (1986) Transport and entrapment of fish larvae by ocean

mesoscale eddies and currents in Hawaiian waters. *Deep Sea Research Part A Oceanographic Research Papers*, **33,** 483-500.

McEdward, L.R. (1986) Comparative morphometrics of echinoderm larvae. II. Larval size, shape, growth, and the scaling of feeding and metabolism in echinoplutei. *Journal of Experimental Marine Biology and Ecology*, **96,** 267-286.

McEdward, L.R. (1986) Comparative morphometrics of echinoderm larvae. I. Some relationships between egg size and initial larval form in echinoids. *Journal of Experimental Marine Biology and Ecology*, **96,** 251-265.

Nei, M. and Li, W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, **76,** 5269-5273.

Nei, M. and Tajima, F. (1981) DNA polymorphism detectable by restriction endonucleases. *Genetics*, **97,** 145-163.

Palumbi, S.R. (1996a) What can molecular genetics contribute to marine biogeography? An urchin's tale. *Journal of Experimental Marine Biology and Ecology*, **203,** 75-92.

Palumbi, S.R. (1996b) Molecular biogeography of the Pacific. *Coral Reefs*, **16S,** 1-2119.

Palumbi, S.R., Grabowsky, G., Duda, T., Geyer, L. and Tachino, N. (1997) Speciation and population genetic structure in tropical Pacific sea urchins. Evolution, 51, 1506-1517.

Palumbi, S.R. (2003) Population genetics, demographic connectivity, and the design of

marine reserves. *Ecological Applications*, **13,** 146-158.

Palumbi, S.R. and Wilson, A.C. (1990) Mitochondrial DNA diversity in the sea urchins Strongylocentrotus purpuratus and S. droebachiensis. *Evolution*, **44,** 403-415.

Paulay, G. and Meyer, C. (2002) Diversification in the tropical Pacific: comparisons between marine and terrestrial systems and the importance of founder speciation. *Integrative and Comparative Biology*, **42,** 922-934.

Peakall, R.O.D. and Smouse, P.E. (2006) Genalex 6: genetic analysis in Excel: population genetic software for teaching and research. *Molecular Ecology Notes*, **6,** 288-295.

Pritchard, J.K., Stephens, M. and Donnelly, P. (2000) Inference of population structure using multilocus genotpye data. *Genetics*, **155,** 945-959.

Scheltema, R.S. (1971) Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. *Biological Bulletin*, **140,** 284-322.

Selkoe, K.A. and Toonen, R.J. (2010) Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. *Marine Ecology Progress Series*,

Shanks, A.L., Grantham, B.A. and Carr, M.H. (2003) Propagule dispersal distance and the size and spacing of marine reserves. *Ecological Applications*, **13,** 159-169.

Skillings, D., Bird, C.E. and Toonen, R.J. (2011) Gateways to Hawaii: genetic population

structure of the tropical sea cucumber *Holothuria atra*. *Journal of Marine Biology*, **2011,** 16.

Sotka, E.E., Wares, J.P., Barth, J.A., Grosberg, R.K. and Palumbi, S.R. (2004) Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. *Molecular Ecology*, **13,** 2143-2156.

Sponaugle, S., Cowen, R.K., Shanks, A., Morgan, S.G., Leis, J.M., Pineda, J., Boehlert, G.W., Kingsford, M.J., Lindeman, K.C., Grimes, C. and Munro, J.L. (2002) Predicting self-recruitment in marine populations: Biophysical correlates and mechanisms. *Bulletin of Marine Science*, **70,** 341-375.

Strathmann, R.R. and Strathmann, M.F. (1985) "Evolutionary opportunities and constraints demonstrated by artificial gelatinous egg masses" in *Reproduction, Genetics and Distributions of Marine Organisms*. Olsen & Olsen, Fredensborg.

Swearer, S.E., Shima, J.S., Hellberg, M.E., Thorrold, S.R., Jones, G.P., Robertson, D.R., Morgen, S.G., Selkoe, K.A., Ruiz, G.M. and Warner, R.R. (2002) Evidence of self-recruitment in demersal marine populations. *Bulletin of Marine Science*, **70,** 251-271.

Thorson, G. (1950) Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews*, **25,** 1-45.

Timmers, M., Andrews, K., Bird, C.E., deMaintenton, M., Brainard, R. and Toonen, R.J. (2011) Widespread dispersal of the crown-of-thorns sea star, *Acanthanster planci*, across the Hawaiian Archipelago and Johnston Atoll.

Titcomb (1978) Native Use of Marine Invertebrates in Old Hawaii. *Pacific Science*, **32,** 325-386.

Toonen, R.J., K. R. Andrews, I.B. Baums, *et al*. (2011) Defining boundaries for ecosystem-based management: a multispecies case study of marine connectivity across the Hawaiian Archipelago. *Journal of Marine Biology*, **2011,** 1-13.

Travis, S.E., Maschinski, J. and Keim, P. (1996) An analysis of genetic variation in *Astragalus cremnophylax var. cremnophylax*, a critically endangered plant, using AFLP markers. *Molecular Ecology*, **5,** 735-745.

Uthicke, S. and Benzie, J.A.H. (2003) Gene flow and population history in high dispersal marine invertebrates: Mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata: Holothuroidea) populations from the Indo-Pacific. *Molecular Ecology*, **12,** 2635-2648.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T.v.d., Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M. and Zabeau, M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23,** 4407-4414.

Weersing, K. and Toonen, R.J. (2009) Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series*, **393,** 1-12.

White, C., Selkoe, K.A., Watson, J., Siegel, D.A., Zacherl, D.C. and Toonen, R.J. (2010) Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B*, **277,** 1685-1694.

Williams, S.T. and Benzie, J.A.H. (1997) Indo-West Pacific patterns of genetic

differentiation in the high-dispersal starfish *Linckia laevigata*. *Molecular Ecology*, **6,** 559-573.

Yasuda, N., Nagai, S., Hamaguchi, M., Okaji, K., Gerard, K. and Nadaoka, K. (2009) Gene flow of Acanthaster planci (L.) in relation to ocean currents revealed by microsatellite analysis. *Molecular Ecology*, **18,** 1574-1590.

Yu, D.H. and Chu, K.H. (2006) Low genetic differentiation among widely separated populations of the pearl oyster *Pinctada fucata* as revealed by AFLP. *Journal of Experimental Marine Biology and Ecology*, **333,** 140-146.

Zeigler, A. C. (2002) *Hawaiian Natural History, Ecology and Evolution*, University of Hawaii Press, Honolulu, Hawaii.

Zhao, Y., Li, Q., Kong, L., Bao, Z. and Wang, R. (2007) Genetic diversity and divergence among clam *Cyclina sinensis* populations assessed using amplified fragment length polymorphism. *Fisheries Science*, **73,** 1338-1343.

Zhivotovsky, L.A. (1999) Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology*, **8,** 907-913.