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Research Article

Gateways to Hawai'i: Genetic Population Structure of the Tropical Sea Cucumber *Holothuria atra*

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Holothuria atra is one of the most common and widest ranging tropical, coral reef sea cucumbers in the world, and here we examine population genetic structure based on mitochondrial COI to aid in determining the appropriate scale for coral reef management. Based on SAMOVA, AMOVA and BARRIER analyses, we show that despite its large range, *H. atra* has hierarchical, fine-scale population structure driven primarily by between-archipelago barriers, but with significant differences between sites within an archipelago as well. Migrate analyses along with haplotype networks and patterns of haplotype diversity suggest that Hawai'i and Kingman reef are important centers of the genetic diversity in the region rather than an evolutionary dead-end for migrants from the Indo-Pacific. Finally we show that for *H. atra* Kingman Reef is the most likely stepping stone between Hawai'i and the rest of the Pacific, not Japan or Johnston Atoll as previously presumed. Based on our data, Johnston Atoll can instead be seen as an outpost of the Northwestern Hawaiian Islands rather than a gateway to the Hawaiian Archipelago.

1. Introduction

Echinoderms play a major role in structuring many marine ecosystems, and many are described as "keystone species" because of their profound influence on benthic community structure (e.g., [1–3], reviewed by Uthicke et al. [4]). In addition to their important ecosystem functions, many echinoderm species are also the focus of artisanal or commercial fishing efforts, particularly the sea urchins and sea cucumbers [5–7]. The influence of echinoderm harvest on a wide range of other commercial fisheries, such as abalone, lobster, kelp, and kelp-associated fin fish, has long stimulated discussions of multispecies approaches to managing their exploitation (e.g., [5], reviewed by Purcell [7]). Delineation of the appropriate spatial scales for management zones within a spatial management network requires a detailed understanding of dispersal pathways and population connectivity (reviewed by Hedgecock et al. [8], Thorrold et al. [9], Fogarty and Botsford [10]).

Understanding connectivity in the sea is complicated by the fact that many marine organisms share a biphasic life cycle typified by an adult form that is relatively sedentary and a larval form that can potentially disperse across large expanses of open ocean [11–15]. For example, in the sea urchin genus *Tripneustes,* some well-known biogeographic barriers, such the Isthmus of Panama or the long stretch of deep water in the western Atlantic, are important barriers to dispersal whereas others, such as the Eastern Pacific Barrier, show no evidence for limiting dispersal [16]. However, the geographic limits of such dispersal are uncertain because it is virtually impossible with current technology to directly track these microscopic juveniles during the pelagic phase (reviewed by Levin [17]) making indirect methods of quantifying larval dispersal particularly attractive (reviewed by Hedgecock et al. [8], Grosberg and Cunningham [18], Selkoe et al. [19], and Hellberg [20]). Proxies for dispersal, such as pelagic larval duration (PLD) and geographic range, have generally been used as rules of thumb in the absence of a detailed understanding of connectivity for most marine species. Unfortunately, intuitive expectations of larval dispersal potential as a function of PLD and

range size are not upheld in recent meta-analyses of the existing literature [21–26, 88]. Realized dispersal distance is typically less than potential dispersal distance because of the presence of biophysical or biogeographical barriers [27–31]. Barriers that limit dispersal between marine populations include obvious geographical features such as land masses like the Isthmus of Panama [32], but also more subtle factors such as currents and oceanographic regimes [33–37]. The correlation between geographic distance and the probability of larval exchange among sites is low in many marine systems (e.g., [38]), and thus quantitative estimates of connectivity are an important prerequisite for delineating the appropriate scale over which marine populations ought to be managed.

The Hawaiian Archipelago lies at the periphery of the tropical Central Pacific and is the most isolated island chain in the world, making it biogeographically partitioned from the rest of the Pacific Islands (reviewed by Ziegler [39]). This isolation results in one of the highest proportions of endemism in the world (e.g., [40–42]; reviewed by Ziegler [39], Eldredge and Evenhuis [43]). Though there are many examples of pan-pacific coral reef organisms in Hawai'i, the isolation of the Hawaiian Archipelago is thought to limit larval exchange sufficiently that colonization is rare [44]. For example, Kay [45] estimated that Western Pacific marine species successfully colonize the Hawaiian Archipelago about once every 13,000 years. Unlike the terrestrial fauna, however, the Hawaiian marine fauna contains a large proportion of endemics that are differentiated but not diversified from their Indo-West Pacific roots [39, 46–48]. Johnston Atoll is believed to be a stepping stone into Hawai'i, and simulations of larval dispersal suggest that larvae from Johnston atoll can reach French Frigate Shoals or Kaua'i along two separate larval corridors [49, 50].

The lollyfish, *Holothuria atra*, is one of the most common shallow-water tropical sea cucumbers in the Indo-Pacific, spanning from Madagascar to French Polynesia [51, 52]. *H. atra* performs vital ecosystem services on coral reefs for which there is an active fishery in many regions of the Pacific [7, 53, 54]. Echinoderms are described as a boom-bust phylum in which populations go through marked natural population cycles [4], an attribute that can compound problems in a harvested population but may hasten repopulation in previously impacted areas. As such, there is a call for ecosystem-based management of sea cucumber harvests [7]. Furthermore, the boom-bust nature of echinoderms has important implications for connectivity in evolutionary time frames, where biological attributes can drive population structure to a greater extent than oceanographic processes as hypothesized in the *Tripneustes* sea urchins [16]. Together these characteristics make *H. atra* an ideal organism to examine levels of connectivity and historical population dynamics to inform management and to test hypotheses about Hawai'i's connection with other archipelagos in the Central Pacific. Here, we assess the inferred range of dispersal for *H. atra* in Hawai'i and the Central Pacific by investigating its mitochondrial genetic population structure in an attempt to delineate the appropriate scales for management.

2. Methods

2.1. Sampling, PCR, and Sequencing. *Holothuria atra* was sampled from five archipelagos (Hawaiian Islands, Line Islands, Marshal Islands, Bonin Islands, and Ryukyu Islands) at a total of 19 sites (Figure 1). Sampling in the Northwest Hawaiian Islands and the Line Islands took place on research cruises aboard the NOAA *R.V. Hi'ialakai*. All other samples were collected on shore dives or while snorkeling. Sampling took place between spring 2006 and fall 2009. Samples were obtained nonlethally through muscle-tissue biopsy and preserved in either 95% ethanol or DMSO salt buffer and archived at the Hawai'i Institute of Marine Biology at room temperature. Skillings and Toonen [55] made an extended discussion of sampling and preservation protocol. No asexual morphs—distinguished by transverse scarring, smaller body size, and their location in lagoonal habitats—were found during sampling expeditions, and no reports are known indicating the presence of the asexual stage of *H. atra* in the sampled locations. The asexual morph of *H. atra* appears to be located only in the Southern and West Pacific (e.g., [52, 56, 57]).

Total genomic DNA was extracted using DNeasy Blood and Tissue Kits (QIAGEN) following the manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify a 423-base pair fragment of the mitochondrial *cytochrome c oxidase subunit I* gene (COI) using custom primers created with Primer3 [58] targeting *Holothuria* spp.: GenHol2L (5′-AACCAAATGGTTCTTGCTTACC-3′) and GenHol2R (5′-TTCTGATTAATCCCACCATCC-3′). PCR was performed using 15 μL reactions containing 1 μL of diluted DNA extract (one part template DNA to 199 parts nanopure water), 1 μL each of 0.2 μM forward and reverse primers, 0.6 μL of 0.5 μM BSA, 7.5 μL of (Bioline) Biomix Red diluted as per manufacturer's instructions, and 3.9 μL of nanopure water. PCR was done on Icycler thermocyclers (Bio-Rad Laboratories) with an initial denaturation at 95°C for 7 min followed by 35 cycles of a denaturing step at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min. A final extension at 72°C was held for 7 min before refrigeration. PCR product (8 μL) was treated with 0.7 μL of *Exonuclease I* combined with 0.7 μL of calf intestinal alkaline phosphatase (Exo-CIAP) and incubated at 37°C for 30 minutes and with a final inactivation step at 85°C for 10 minutes. The treated PCR product was sequenced using an ABI Prism automatic sequencer at the Hawai'i Institute of Marine Biology's EPSCoR sequencing facility. All samples were sequenced in the forward direction; uncertain sequences and all unique haplotypes were also sequenced in the reverse direction for confirmation. Sequences were compiled and trimmed using Sequencher 4.8 and aligned using ClustalW implemented in Bioedit 7.0.5 [59, 60].

2.2. Data Analysis. A statistical parsimony network of mitochondrial haplotypes was constructed by creating a reduced median network that was then used to make a median joining network; both procedures implemented in Network 4.516 (http://www.fluxus-engineering.com/; [61,

62]). The network was drawn using Network Publisher 1.1.0.7 (http://www.fluxus-engineering.com/).

Nei's average pairwise genetic difference (π) [63] and haplotype diversity (h) were calculated in DnaSP 4.1 [64]. The effective number of alleles was calculated by hand following Jost [65]. Tajima's D [66] and Fu's F_S [67] were calculated in Arlequin v.3.1 with 10,000 simulations to establish confidence intervals.

To assess levels of genetic differentiation between sites, we calculated pairwise Φ_{ST} values using Arlequin 3.1 [68] and pairwise D_{est_chao} values by hand following Jost [65]. Φ_{ST} is a fixation index incorporating genetic distance that ranges from 0 to 1, where a zero indicates identical haplotypic composition and a one signifies alternate fixation of alleles and a complete lack of gene flow. D_{est_chao} is an index of genetic differentiation, which does not account for genetic distance among haplotypes but also ranges from 0 to 1 (note that both Φ_{ST} and D_{est_chao} can be slightly negative due to bias correction for sampling error). In the case of D_{est_chao}, a zero also indicates identical haplotypic composition, but unlike Φ_{ST}, a one simply indicates that no haplotypes are shared between the populations. The primary difference in interpretation is that in the absence of gene flow Φ_{ST} values can be significantly less than one, while this is not the case for D_{est_chao}, which is argued to be an advantage of this latter statistic [65]. To correct the critical P value for statistical significance in pairwise comparisons, the familywise false discovery rate (FDR) correction found in [69] was implemented. Analysis of molecular variance (AMOVA) was used for hierarchical analysis of the partitioning of COI diversity among sites within archipelagic regions and among archipelagic regions using Arlequin 3.1. SAMOVA 1.0 was used to identify groups of samples that maximize the proportion of total genetic variance due to differences between regions [70]. The most important genetic barriers were ranked using BARRIER 2.2 [71]. BARRIER uses Monmonier's maximum-difference algorithm to compare a matrix of difference values, such as pairwise Φ_{ST} values, with a matrix of geographic distances in order to identify the strongest barriers within the matrix. We compared barriers created using each Φ_{ST} and D_{est_chao} distance matrix. AMOVAs were performed using groupings determined by SAMOVA and BARRIER for hypothesis testing to compare genetic groupings to the archipelagic groupings. The pairwise Φ_{ST} and AMOVA analyses were conducted using a distance matrix with 50,000 permutations and the Tamura-Nei mutational model [72] with gamma = 0.0164. The mutational model HKY+G was selected using AIC in MODELTEST 3.7; the model hierarchy was used to select the closest available model when the best-fit model could not be implemented by the chosen program, as in the case of ARLEQUIN [73]. Regardless, the inferences are robust to the mutational model, and our conclusions are not altered regardless of which model is chosen (data not shown).

MRBAYES 3.1 was used to construct a Bayesian estimation of a phylogeny containing all *H. atra* haplotypes from this study along with all *Holothuria* and *Actinopyga* COI haplotypes available from GenBank as of February 2010 [74]. Two independent runs with identical conditions were completed and averaged. A general time reversible (GTR) simple nucleotide model with a gamma-shaped rate variation of 0.0164 was used; Markov chain length = 4 × 3,000,000 sampled every 100 generations with a 10% burn-in. The GTR nucleotide model was chosen as it is the most general and neutral nucleotide model available in MrBayes 3.1 and corresponds most closely to the Tamura-Nei model [75]. The sea cucumber *Actinopyga agassizi* was set as the outgroup. Program defaults were used for all other settings. MrBayes was used to summarize all of the trees produced into a single consensus tree.

RAxML 7.0 [76] implemented through CIPRES Web Portal v.1.15 [77] was used to construct the highest scoring maximum likelihood-based estimation of a phylogeny containing all haplotypes used in the Bayesian analysis and run 10,000 bootstrap simulations to assess branch support. A GTR nucleotide model that uses four discrete gamma rates set by the program was used for the analysis; program defaults were used for all other settings.

Bayesian coalescent-based calculations of migration rate among regions (N_eM) and the region mutation parameter (θ) were conducted using MIGRATE 3.1.3 [78]. Three independent runs of a Bayesian MCMC search strategy were completed and averaged by MIGRATE. A nucleotide model with a transition-to-transversion ratio of 6.1584 : 1 and three regions of substitution rates with a gamma-shaped rate variation of 0.016 was used; Markov chain length = 1,000,000 sampled every 20 generations with a 10% burn-in. Program defaults were used for all other settings. The transition-to-transversion ratio was calculated using Modeltest 3.7. Two replicate MIGRATE analyses were run using different population groupings. Preliminary analyses that split the data by sampling location returned flat posterior probabilities, presumably from having too many parameters to estimate. The software's author advocates using the minimal number of sensible regions in order to reach convergence (Peter Beerli, pers. comm.). The first analysis used regions separated along the most important breaks identified by the program BARRIER. Archipelagos were used as regions for the second analysis with the Hawaiian Archipelago further divided between the main Hawaiian Islands and the Northwestern Hawaiian Islands plus Johnston Atoll and Kingman Reef separated from the Line Islands; this grouping was chosen based on BARRIER and pairwise analyses. Values for the migration rate among regions (M) and region mutation parameters (θ) were taken from the highest peaks in the posterior probability distribution curves. The posterior probability distributions were examined to determine the validity of each estimated parameter.

3. Results

A total of 385 individuals, 55 haplotypes, and 37 private haplotypes are sampled in this study (Table 1). Of the 18 haplotypes shared across sampling sites, 10 are found in multiple archipelagos (Figure 2). However, no haplotypes are shared between the most distant regions: Hawai'i and Japan.

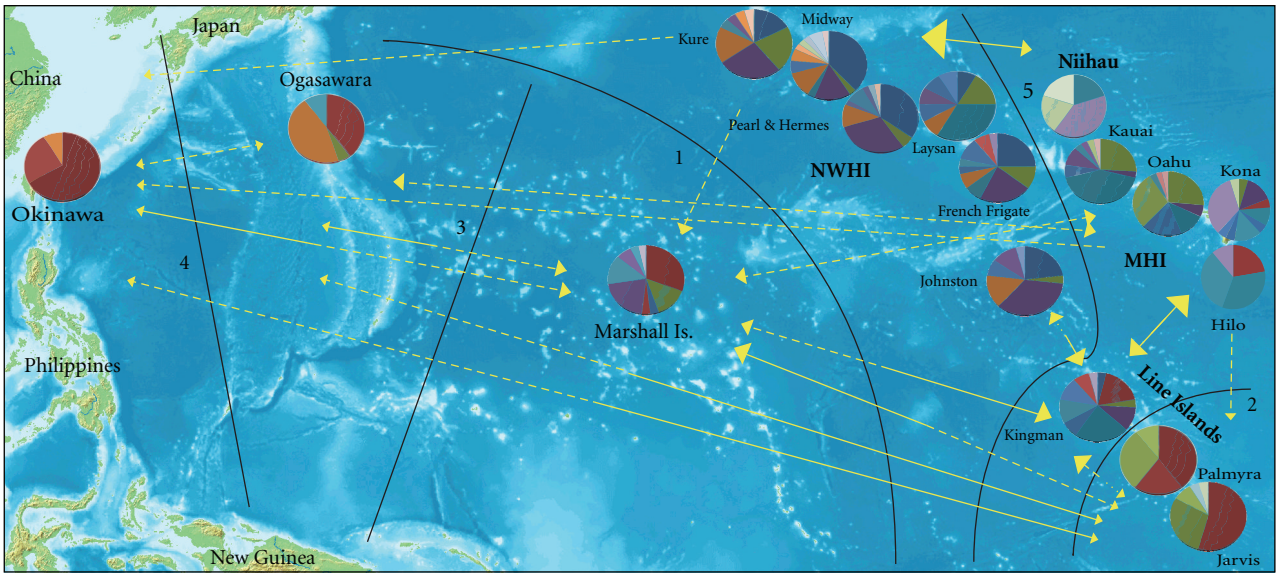

FIGURE 1: Map of the northern Central and West Pacific. Pie charts represent haplotype frequencies overlaid on sites. To avoid overlap, the Marshall Islands sites, Kwajalein and Majuro, have been combined. Black lines are major gene flow constraints drawn by BARRIER, numbered from strongest to weakest. Yellow arrows represent directional migration rates estimated by MIGRATE. Effective migration rate estimates (N_eM) with modes between 0.01 and 0.49 are represented by small arrows, rates between 0.5 and 0.99 are represented by medium arrows, and rates over 1.0 are represented by large arrows. Solid lines represent migrate posterior probability distributions, where the 50% credibility set does not include zero. Dashed lines represent migration posterior probability distributions, where the 50% credibility set includes zero, but the distribution peak is greater than zero. Migration parameters with a posterior distribution peak of zero are not shown.

Because many population genetic estimates are relatively insensitive to weak selection [79], loci which do not show significant deviations from neutral expectations should provide reliable inferences about population structure [80]. None of the site-by-site Tajima's D values were significant, and only Laysan deviated from expectation using Fu's F_s; thus, there is no evidence to indicate that nonneutral processes are responsible for the pattern of COI haplotype diversity presented here.

To ensure there was no misidentification of the samples included here, we performed a phylogenetic reconstruction of our samples with those available in GenBank. We confirmed that the samples included here are monophyletic and there are no reciprocally monophyletic groups among the *H. atra* haplotypes included in our population genetic analyses (Appendix A).

Haplotype diversity, as a function of longitude, increases from west to east across the Pacific Ocean (Table 1, $R^2 = 0.80$, $P < 0.05$). Japanese sites exhibit the lowest haplotype diversity ($h = 0.51$–0.66) and effective number of haplotypes ($H_E = 2.0$–2.9); the centrally located Line Islands ($h = 0.65$–0.90, $H_E = 2.9$–9.7) and Marshall Islands ($h = 0.75$–0.76, $H_E = 4.0$–4.1) exhibit mid to high levels of diversity; the Hawaiian sites exhibit the highest diversity ($h = 0.75$–0.90, $H_E = 4$–10). Nucleotide diversity does not appear to be correlated with haplotype diversity because nucleotide diversity is lower in the Hawaiian Archipelago than in all other locations except for Okinawa (Table 1). This pattern can be visualized in the haplotype network, where sites exhibiting high nucleotide diversity harbor disparate haplotypes separated by a relatively large number of mutations (Figure 2).

Four AMOVAs were run on the *H. atra* COI haplotype data (Table 2). In each AMOVA, a different method was employed to group the population samples into regions. Under the first geographic hypothesis, sites were grouped into five regions by archipelago, with Johnston Atoll included with Hawai'i. In order to assess the subdivision of the Hawaiian Archipelago into the NWHI and the MHI, this grouping hypothesis was compared to a second geographic hypothesis, where population samples were grouped into six regions; Hawaiian sites were divided into the MHI and the NWHI + Johnston and four regions comprised of the four remaining archipelagos. Both grouping hypotheses (geographic hypothesis one and two) exhibited similar partitioning of variation among groups (32.1% versus 30.9%) and among populations within groups (8.9% versus 5.5%). In both cases, there was stronger partitioning among the groups of samples ($\Phi_{CT} = 0.32$, $\Phi_{CT} = 0.31$; $P < 0.0001$) than among the simples nested within the groupings ($\Phi_{SC} = 0.13$, $\Phi_{SC} = 0.07$; $P < 0.0001$).

The two geographic hypotheses were compared to six-region groupings identified by SAMOVA and BARRIER; BARRIER selected the same dominant barriers using both the Φ_{ST} and D_{est_chao} distance matrices (Table 2 and Figure 1). Six regions were chosen for a direct comparison to the archipelagic geographic hypothesis that included Hawai'i divided into two main regions. These groupings partitioned variance similarly to the geographic hypotheses with the SAMOVA grouping minimizing among-population within-group variance with more among-group variance explained (Table 2). The grouping of population samples using BARRIER and SAMOVA had slightly greater levels of genetic

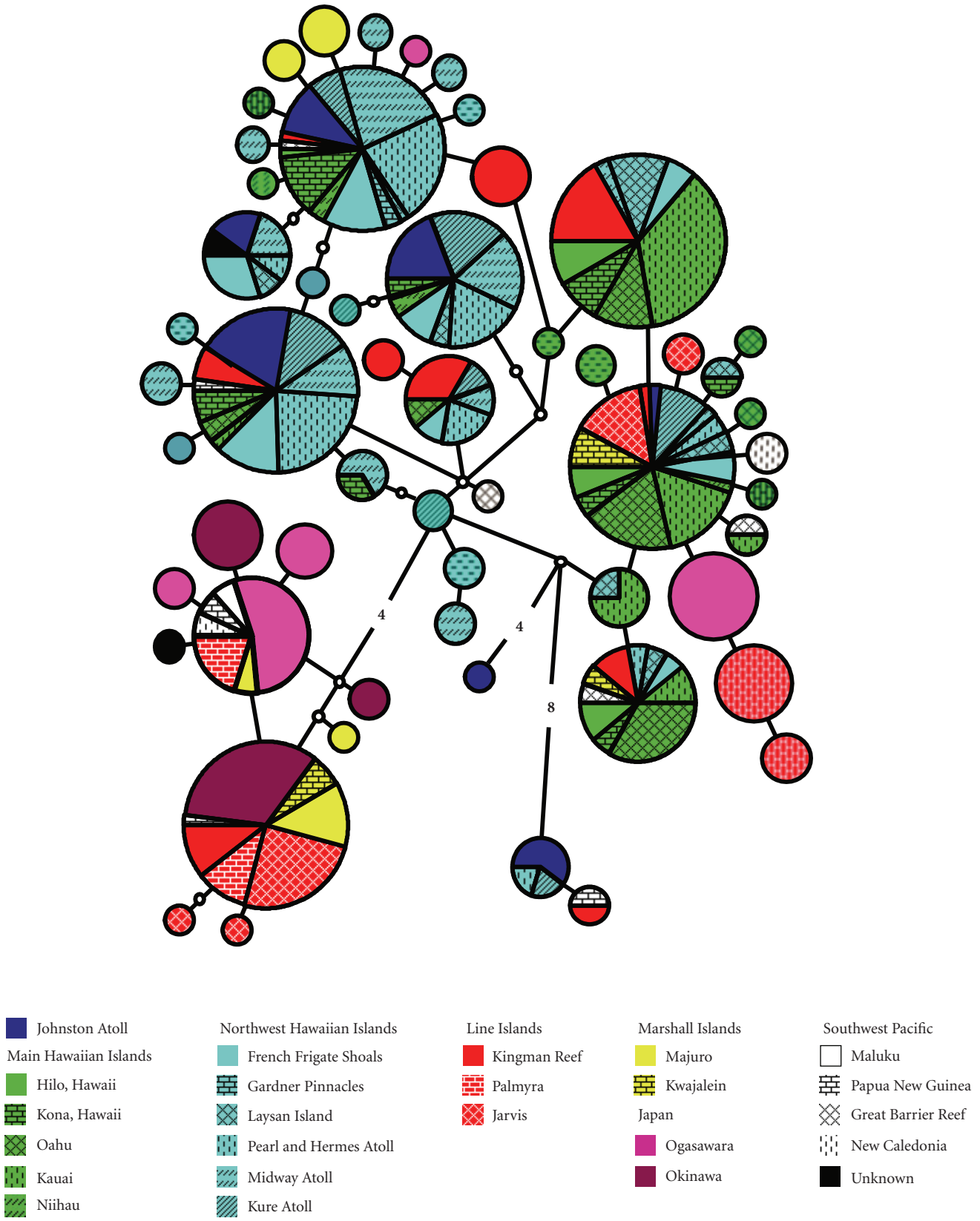

Figure 2: Haplotype network for *Holothuria atra*. Each circle represents a unique haplotype connected by a line to those that differ by one base pair. Nodes on lines indicate a missing haplotype, and numbers represent multiple missing haplotypes. Each haplotype is color coded by site, and circle size is proportional to frequency. The smallest circles represent one occurrence of a halotype.

Table 1: N is the Sample size, H is the total number of haplotypes, H_u is the number of unique haplotypes at site, π is the nucleotide diversity, h is the haplotype diversity, and A_E is the effective number of alleles in COI.

Region	Site	N	H	H_u	$\pi \pm$ SD	$h \pm$ SD	A_E	Tajima's D	Fu's F_s
Main Hawaiian Islands	Hilo	9	4	0	0.0041 ± 0.0030	0.81 ± 0.08	5.3	−0.27	0.08
	Kona	21	10	2	0.0078 ± 0.0046	0.87 ± 0.06	7.5	−0.57	−2.21
	Oahu	24	7	2	0.0052 ± 0.0033	0.79 ± 0.05	4.7	−0.88	−0.58
	Kauai	30	8	2	0.0033 ± 0.0023	0.75 ± 0.06	4.0	−1.21	−2.61
	Niihau	5	4	1	0.0071 ± 0.0052	0.90 ± 0.16	10.0	−0.75	−0.33
Northwest Hawaiian Islands	French Frigate	28	10	2	0.0082 ± 0.0048	0.88 ± 0.04	8.3	−0.14	−1.12
	Gardner	2	1	0	N/A	N/A	N/A	N/A	N/A
	Laysan	12	8	0	0.0064 ± 0.0041	0.89 ± 0.08	9.1	−1.06	**− 2.91**
	Pearl & Hermes	37	10	2	0.0086 ± 0.0049	0.79 ± 0.05	4.8	−1.26	−0.23
	Midway	35	14	6	0.0084 ± 0.0048	0.84 ± 0.05	6.2	−0.64	−3.61
	Kure	23	8	2	0.0096 ± 0.0055	0.85 ± 0.04	6.9	−0.92	0.47
Johnston	Johnston	26	7	1	0.0131 ± 0.0073	0.81 ± 0.05	5.3	−0.28	2.96
Line Islands	Kingman	27	10	3	0.0142 ± 0.0078	0.90 ± 0.03	9.7	−0.39	0.81
	Palmyra	16	4	2	0.0155 ± 0.0087	0.76 ± 0.06	4.1	2.59	6.13
	Jarvis	23	5	3	0.0126 ± 0.0070	0.65 ± 0.08	2.9	1.8	4.84
Marshall Islands	Majuro	14	5	3	0.0139 ± 0.0079	0.76 ± 0.08	4.1	2.22	3.32
	Kwajalein	9	4	1	0.0126 ± 0.0076	0.75 ± 0.11	4.0	1.49	2.81
Bonin Islands	Ogasawara	20	4	3	0.013 ± 0.0073	0.66 ± 0.06	2.9	2.25	6.15
Ryukyu Islands	Okinawa	24	3	2	0.0028 ± 0.0021	0.51 ± 0.09	2.0	0.26	1.82
Overall		385	55	37	0.0088 ± 0.0045	0.92 ± 0.08	12.5	0.12 ± 1.28	0.83 ± 2.91

differentiation among groups of samples ($\Phi_{CT} = 0.33$, $\Phi_{CT} = 0.37$; $P < 0.0001$) and lower levels of differentiation among samples nested within groups ($\Phi_{SC} = 0.07$, $\Phi_{SC} = 0.03$; $P < 0.0001$). Overall, the four AMOVAs exhibited similar levels of partitioning of variance, and all tests were significant ($P < 0.0001$).

There is a strong pattern of restricted gene flow between sites among the population samples of *H. atra*. Pairwise comparisons for both Φ_{ST} and D_{est_chao} reveal significant differences between sites located in different archipelagos in almost all cases, where sample sizes are 10 or greater (Table 3). The exceptions are between the Line Islands and the Marshall Islands, where one of six pairwise Φ_{ST} comparisons is statistically significant; between the Line Islands and the Bonin Islands, one of three Φ_{ST} comparisons is statistically significant; between the Hawaiian Islands and Kingman Reef, where only four of twelve pairwise Φ_{ST} comparisons were statistically significant.

Some significant differences were also detected among samples within archipelagos. In the Main Hawaiian Islands (MHI), O'ahu and Kaua'i are significantly different than the Kona sample from the Big Island of Hawai'i. Despite small sample sizes, Ni'ihau is also partitioned from the adjacent island of Kaua'i as well as O'ahu, but not the Big Island. Within the Northwestern Hawaiian Islands (NWHI), only Laysan is significantly partitioned from the other sampling sites, including Johnston Atoll. Overall, 42% of pairwise comparisons between the MHI and the NWHI + Johnston were significant, compared to 30% of the comparisons within the MHI and 19% of the comparisons within the NWHI + Johnston. The samples from both the Line Islands (LI) and the Marshall Islands (MI) were significantly partitioned within their respective archipelagos when using D_{est_chao}; there was not significant partitioning between MI sites when using Φ_{ST}.

The results from the MIGRATE runs show similar patterns of gene flow between regions (Table 4, Figure 1). Effective migration rates (N_eM) between regions are low. There is less than one migrant per generation, the rule-of-thumb number below which population cohesion starts to break down, between most regions [81]. The exceptions include the one-way migration from the Main Hawaiian Islands into the Northwest Hawaiian Islands and Johnston Atoll and the one-way migration from Kingman Reef to the Main Hawaiian Islands (Table 4). The high effective migration rate from the NWHI to the MHI in the first analysis splits almost evenly between Kingman Reef and the MHI when these two regions are separated in the second analysis (Table 4). Overall, higher effective migration rates are observed leaving the Hawaiian regions then going into them (Table 4, Figure 1). Though effective migration rates are a product of migration and effective population size, the effective migration rates larger than 1 migrant per generation are driven primarily by migration and not effective population size (Appendices A and B). This pattern is indicative of recent migration rather than ancestral polymorphisms and high effective population sizes. Posterior probability distributions for all values were in the form of unimodal curves. A full description of N_e and M values for both analyses can be found in Appendices B and C.

Table 2: Pairwise comparisons by site. D_{est_chao} values are contained in the lower left half of the table, and Φ_{st} values are in the upper right half of the table. Bolded values signify significant differences after correction using the procedure outlined in Benjamini 2008.

Region	Site	Main Hawaiian Islands					Northwest Hawaiian Islands						Johnston	Line Islands			Marshall Islands		Bonin Iss.	Ryukyu Iss.
		Hilo	Kona	Oahu	Kauai	Niihau	Fr. Frigate	Gardner	Laysan	Pearl &Hermes	Midway	Kure		Kingman	Palmyra	Jarvis	Majuro	Kwajalein	Ogasawara	Okinawa
Main Hawaiian Islands	Hilo	—	0.101	−0.039	−0.029	**0.234**	**0.209**	0.463	−0.059	**0.253**	**0.243**	**0.185**	**0.204**	0.084	**0.303**	**0.399**	**0.416**	0.159	**0.335**	**0.864**
	Kona	0.225	—	**0.127**	**0.203**	−0.047	−0.006	−0.044	0.049	0.026	0.016	0.009	0.046	0.056	**0.327**	**0.401**	**0.361**	**0.203**	**0.345**	**0.786**
	Oahu	−0.167	**0.515**	—	0.049	**0.261**	**0.221**	**0.399**	0.018	**0.258**	**0.263**	**0.193**	**0.231**	**0.117**	**0.351**	**0.431**	**0.468**	**0.181**	**0.368**	**0.826**
	Kauai	−0.109	**0.499**	0.172	—	**0.377**	**0.305**	**0.568**	0.001	**0.341**	**0.335**	**0.278**	**0.309**	**0.183**	**0.456**	**0.524**	**0.573**	**0.333**	**0.473**	**0.873**
	Niihau	0.245	−0.388	0.516	**0.698**	—	−0.011	−0.224	0.091	0.006	−0.045	−0.005	0.007	0.024	0.266	0.359	0.269	0.178	0.301	**0.847**
Northwest Hawaiian Islands	Fr. Frigate	0.392	−0.105	**0.518**	**0.629**	−0.239	—	−0.039	**0.145**	−0.014	−0.012	0.001	0.015	**0.084**	**0.358**	**0.421**	**0.367**	**0.256**	**0.371**	**0.767**
	Gardner	0.814	0.412	**1**	**1**	0.273	0.553	—	0.23924	−0.021	−0.091	0.033	−0.045	0.011	0.258	**0.375**	0.215	0.199	**0.315**	**0.889**
	Laysan	−0.294	0.138	0.122	−0.13	0.191	0.286	0.849	—	**0.194**	**0.173**	**0.124**	**0.156**	0.068	**0.306**	**0.396**	**0.398**	0.167	**0.334**	**0.832**
	Pearl & Hermes	**0.689**	0.008	**0.744**	**0.888**	−0.11	−0.033	0.42	**0.674**	—	−0.012	−0.013	−0.005	**0.089**	**0.365**	**0.419**	**0.358**	**0.269**	**0.372**	**0.739**
	Midway	**0.661**	−0.08	**0.846**	**0.88**	−0.356	−0.026	0.36	**0.555**	−0.018	—	0.004	0.0177	0.091	**0.365**	**0.421**	**0.349**	**0.271**	**0.373**	**0.747**
	Kure	0.461	0.111	**0.41**	**0.666**	−0.201	−0.03	0.697	**0.483**	0.0349	0.16	—	−0.013	0.042	**0.304**	**0.369**	**0.329**	**0.197**	**0.316**	**0.739**
	Johnston	**0.799**	0.144	**0.782**	**0.901**	0.0909	−0.012	0.611	**0.694**	−0.041	0.093	−0.022	—	0.071	**0.297**	**0.358**	**0.294**	**0.205**	**0.308**	**0.682**
Line Islands	Kingman	0.279	**0.432**	**0.467**	**0.354**	**0.781**	**0.463**	**0.933**	0.142	**0.643**	**0.695**	**0.612**	**0.666**	—	**0.145**	**0.183**	**0.153**	0.019	**0.152**	**0.571**
	Palmyra	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0.664**	–	0.065	0.109	0.031	0.009	**0.451**
	Jarvis	**0.626**	**0.88**	**0.591**	**0.73**	**0.728**	**0.86**	**1**	**0.776**	**0.941**	**0.966**	**0.732**	**0.956**	**0.521**	**0.447**	—	0.041	0.046	0.064	**0.289**
Marshall Islands	Majuro	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0.539**	**0.391**	**0.241**	—	0.099	0.107	**0.386**
	Kwajalein	0.222	**0.752**	0.155	**0.499**	0.492	**0.72**	1	0.532	**0.883**	**0.938**	**0.512**	**0.922**	**0.51**	**0.576**	−0.034	**0.419**	—	0.044	**0.619**
Bonin Islands	Ogasawara	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0.743**	**1**	**0.902**	**1**	—	**0.428**
Ryukyu Islands	Okinawa	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0.585**	**0.433**	**0.172**	**0.222**	**0.402**	**1**	—

Familywise false discovery rate corrected P-values: D_{est_chao} $P \leq .036$; Φ_{st} $P \leq .023$.

Table 3: Analysis of molecular variance (AMOVA) for different population groupings. Beginning at the top, groupings are as follows: (1) by archipelago, (2) by archipelago with Hawaii divided between the Northwest Hawaiian Islands (NWHI) and the Main Hawaiian Islands (MHI), (3) six groups chosen by BARRIER and (4) six groups chosen by SAMOVA.

Population groupings	Source of variation	% of Variation	Φ statistics
Okinawa; Ogasawara; Marshall	AG	32.14	$\Phi_{CT} = 0.321^*$
Islands; Hawaiian Archipelago +	AP(G)	8.9	$\Phi_{SC} = 0.131^*$
Johnston Atoll; Line Islands	WP	58.96	
Okinawa; Ogasawara; Marshall	AG	30.94	$\Phi_{CT} = 0.309^*$
Islands; MHI; NWHI + Johnston Atoll;	AP(G)	5.47	$\Phi_{SC} = 0.0793^*$
Line Islands	WP	63.58	
Okinawa; Ogasawara; Marshall	AG	32.59	$\Phi_{CT} = 0.326^*$
Islands; MHI + Kingman; NWHI +	AP(G)	4.48	$\Phi_{SC} = 0.066^*$
Johnston Atoll; Palmyra + Jarvis	WP	62.93	
Okinawa; Ogasawara + Kwajalein +	AG	37.35	$\Phi_{CT} = 0.373^*$
Palmyra; Jarvis + Majuro; Hilo +	AP(G)	2.02	$\Phi_{SC} = 0.032^*$
Oahu + Kauai; All Others	WP	60.63	

AG = Among groups, AP(G) = Among populations within Groups, WP = Within populations. $^*P < 0.0001$.

Table 4: Pairwise population migration rate estimates (N_eM) based on a Bayesian MCMC simulation. Kingman Reef is grouped with the Main Hawaiian Islands in the upper table and treated as a seprate population in the lower table. The value of M calculated by MIGRATE was multiplied by the θ, as calculated by MIGRATE, of the destination population to estimate migration. The estimates of migration are separated by direction; the columns are source populations, and the the rows are sink populations.

Region	Line Islands	MHI+King	NWHI+J	Okinawa	Ogasawara	Marshall Islands	
Line Islands	—	0.13125	0.00375	0.15375	0.16875	0.13875	
MHI+Kingman	0.02375	—	3.01625	0.02375	0.02375	0.45125	
NWHI+Johnston	0.03125	4.34375	—	0.03125	0.03125	0.03125	
Okinawa	0.04625	0.00875	0.02875	—	0.04375	0.04375	
Ogasawara	0.04625	0.01375	0.00125	0.04875	—	0.02875	
Marshall Islands	0.46125	0.55125	0.19125	0.43875	0.39375	—	
	Line Islands	MHI	NWHI+J	Okinawa	Ogasawara	Marshall Islands	Kingman
Line Islands	—	0.04375	0.01125	0.05125	0.05125	0.04875	0.03125
MH Iss.	0.01625	—	0.95875	0.01625	0.27625	0.30875	1.02375
NWHI+Johnston	0.02625	4.96125	—	0.02625	0.02625	0.02625	0.49875
Okinawa	0.04625	0.05125	0.02875	—	0.04375	0.04875	0.04125
Ogasawara	0.04375	0.02625	0.03125	0.04375	—	0.04875	0.02875
Marshall Islands	0.46125	0.43875	0.41625	0.46125	0.39375	—	0.41625
Kingman Reef	0.69375	0.84375	0.80625	0.88125	0.01875	0.73125	—

4. Discussion

In this survey of population genetic structure, we elucidate patterns of connectivity throughout the north-central range of the sea cucumber *Holothuria atra* with a focus on the Hawaiian Archipelago. The Hawaiian Archipelago is highly isolated and also contains one of the highest proportions of endemism in the world (e.g., [40–42]; reviewed by Ziegler [39], Eldredge and Evenhuis [43]). Though there are many pan-pacific marine organisms in Hawai'i, the isolation of the archipelago is thought to limit larval exchange such that colonization is rare but sufficient to maintain species cohesion among these taxa. The Hawaiian marine fauna contains a large proportion of endemics that are differentiated but not diversified from its Indo-West Pacific roots [39, 46–48]. In this scenario, Hawai'i is seen primarily as a dead end, an isolated land mass that does not contribute in a significant way to the overall diversity of the tropical pacific. Counter to the island biogeography hypotheses of Hawaiian diversity, Jokiel and Martinelli [44] proposed the Vortex model of speciation, wherein the stunning biodiversity of the Coral Triangle is a result of centrifugal accumulation of species from the peripheral habitats around the Pacific. Though these two models primarily make predictions about speciation-level processes and do not speak directly to gene flow within a species, they do make opposite claims about the dominant direction of gene flow and dispersal. *H. atra* has a broad species range, extending from the Western Indian Ocean to the Eastern Pacific Ocean, which suggests the capacity for long-distance dispersal; however, populations showed significant population structuring within archipelagos, sometimes across very short oceanic distances. Even so,

hierarchical genetic population structure in *H. atra* gives insight into the phylogeography of the north-central tropical Pacific. Our data test between the divergent hypotheses of whether peripheral archipelagos act as a source of genetic diversity in the Pacific and the likely colonization routes, into and out of, the extremely isolated Hawaiian Archipelago.

4.1. Biogeography and Range Size. If a large species range is a consequence of high dispersal potential, then *H. atra* should have little pronounced population structure, especially across small scales [11, 15, 82]. Indeed, this is the case for many species in the central West Pacific [16, 83–86]. Despite a species range which stretches from the Western Red Sea to the eastern Central Pacific in which *H. atra* is found in almost all shallow tropical habitats, we did not find support for extensive dispersal. The majority of sites from which we sampled *H. atra* were genetically distinct, with some sites less than 75 km apart being among the most distinct in our study (Table 3). These contrasting patterns highlight the dangers of making predictions about population connectivity and diversity based solely on the location and size of a species' range.

The larval life history of *H. atra* is not known exactly, but they require at least 18–25 days to reach competency to settle and are capable of traversing long oceanic distances with sufficient frequency to maintain species cohesion across a very broad geographic range [87]. The obvious question becomes why then is population subdivision found on such small geographic scales (e.g., Kingman Reef and Palmyra Atoll are only 67 km apart)? Counter to intuition, the geographic distance among sites is a poor predictor of the ease with which larvae can disperse among locations; the "oceanographic distance" experienced by larvae between sites is uncorrelated with geographic separation between them [36, 38]. Likewise, recent meta-analyses indicate the relationship between the length of pelagic larval development and dispersal ability is not as tight as has been generally assumed [23–26, 88]. Finally, a broad meta-analysis by Lester et al. [21] indicates that the intuitive relationship between range size and larval dispersal potential is poorly correlated overall but can play an important role in some taxa. Toonen et al. in this issue also show a number of breaks in the Hawaiian Archipelago that are shared by several species and are unexplained solely by appeal to one metric such as range size or larval dispersal potential. Although the mechanism of isolation across small scales remains unknown, our data clearly indicate that *H. atra* is not one of those species for which range size predicts relative dispersal ability.

4.2. Population Structure in the Hawaiian Archipelago and Johnston Atoll. Our mtDNA examination of *Holothuria atra* reveals significant genetic population structure across the surveyed portion of the range. There are two interesting patterns to this structure. Excluding Laysan Island, there are no significant pairwise differences between any other islands in the NWHI (spanning nearly 2000 km), suggesting that the NWHI, excluding Laysan, comprises a single large population. In contrast, there is significant structuring within the MHI (roughly 600 km) and between the NWHI and the MHI. This finding suggests that factors beyond merely geographic distance influence population partitioning.

Johnston Atoll, the nearest neighboring land mass, roughly 860 km south of French Frigate Shoals, is genetically distinct from most of the MHI and Laysan and genetically similar to all of the NWHI except Laysan. It has been suggested that Johnston Atoll acts as a stepping stone into the Hawaiian Islands [89]. Kobayashi [49, 50] used computer simulations to predict two larval transport corridors from Johnston Atoll to the Hawaiian Archipelago: one corridor stretching from Johnston to French Frigate Shoals in the NWHI and one from Johnston to Oʻahu in the MHI. Our data support the predicted larval transport corridor between Johnston Atoll and French Frigate Shoals, but not the corridor predicted between Kauaʻi and Johnston. Additionally, based on our data, Kingman Reef may also be an important stepping stone into and out of Hawaiʻi. The BARRIER analysis shows the division between the NWHIs, including Johnston Atoll, and the MHI to be the strongest barrier to gene flow within the Archipelago (Figure 1). Migration across this barrier is heavily one sided, where migration from the MHI into the NWHI dominates. The effectively one-way migration rates into the NWHI and Johnston Atoll coupled with the strong genetic similarity between Johnston Atoll and the NWHI suggest Johnston Atoll is an isolated outpost of the Northwest Hawaiian Islands, providing support for a vortex model [44] rather than the stepping stone entry into Hawaiʻi [89] for *H. atra*. These data indicate that Johnston Atoll exchanges migrants with Hawaiʻi far more often than its nearest neighbors to the south, and the same can be said for Kingman Reef. This result is particularly surprising because in the case of Kingman Reef, *H. atra* sampled there show greater similarity to populations in Hawaiʻi (roughly 1700 km southwest of Honolulu) than they do to those sampled at Palmyra Atoll, only 67 km away.

4.3. Phylogeographic Relationships between Archipelagos. Counter to conventional wisdom that Hawaiʻi is a passive recipient of rare dispersal from the diverse Pacific, the weight of available evidence, including pairwise Φ_{ST} values, mtDNA phylogeny, BARRIER divisions, and clustering within the haplotype network, provides substantial evidence for the opposite pattern in *H. atra;* Johnston Atoll is an outpost of Hawaiian diversity, and Kingman Reef acts as the primary stepping stone between the Hawaiian Archipelago and the rest of the Pacific. As far as we are aware, this is the first time empirical evidence has been provided for such a pathway. Higher haplotypic diversity in Hawaiʻi and the Line Islands relative to the other archipelagos supports a scenario in which population sizes are far greater, or Hawaiʻi and/or Kingman Reef are the ancestral population in the region. Likewise, the dominant haplotypes found in the Japanese Archipelagos are relatively distantly related and appear derived (Figure 2), suggesting that the western portion of the surveyed range was colonized in at least two separate events (or one of them has gone extinct in Hawaiʻi

and Kingman), one of which did not make it all the way to Okinawa.

Excluding the dense sampling within the Hawaiian Archipelago, virtually all pairwise comparisons between sites are significantly different from each other (Table 3). The few comparisons that were not significant between sites have relatively high pairwise values but low sample sizes, a likely statistical limitation also noted by Bird et al. [90]. Hierarchical population structuring was detected with AMOVA using either D_{est} or Φ_{ST} values; sampling sites within archipelagos are significantly different from each other but are more similar within than between archipelagos. Four of the five most substantial restrictions to gene flow uncovered in this study (and the top ranked by BARRIER) were those between archipelagos; the one exception to this trend being Kingman Reef which is included with the Hawaiian rather than the Line Islands (Figure 1). The AMOVA run using the regions selected by BARRIER was only minimally different than the AMOVA run using regions divided by archipelagos; grouping Kingman Reef with the MHI, as per BARRIER, did explain 1.5% more of the overall variance.

In addition to the distinct archipelagic groupings in the haplotype network (Figure 2), several other patterns are noteworthy here. First, the NWHI and Johnston Atoll haplotypes are clustered together and interspersed whereas the MHI haplotypes are clustered together. Also, the Japanese haplotypes occur in two divergent areas of the network. The Line Island and Marshall Island haplotypes are interdispersed throughout the network, suggesting that these island groups are either mixing or transition zones. Nearly every locality haplotype (those found in only one sampling location) branch off in a starburst pattern from the major haplotypes found primarily in the same archipelago. This pattern is an indication that regional populations have been separated long enough for new haplotypes to arise, and that these new haplotypes are not being spread to other archipelagos by long-distance dispersal. Uniformly low migration rates between archipelagos estimated with MIGRATE support this isolation scenario.

5. Conclusion

Many echinoderm species are the focus of artisanal or commercial fishing efforts, and managing these fisheries requires a detailed understanding of dispersal pathways and population connectivity within a spatial management network. The Hawaiian Archipelago lies at the periphery of the tropical Central Pacific and is the most isolated island chain in the world; the question remains as to why some species maintain connectivity and species cohesion between the Hawaiian Islands and the rest of the Pacific, why some species diverge and become Hawaiian endemics, and why other species with similar inferred dispersal ability fail to colonize the Hawaiian Archipelago at all.

The genetic diversity of COI in *H. atra* across the studied portion of the range presents a complex pattern, but it is not inscrutable. Based on AMOVA, SAMOVA, and BARRIER analyses, it can be seen that population structuring is hierarchical; there are significant differences between sites, but the primary degree of population structure is archipelago by archipelago. Our analyses taken together suggest that the Hawaiian Archipelago and Kingman reef are ancestral populations in the region with migration moving out of these periphery archipelagos toward a less diverse central Pacific rather than the reverse. This pattern is inconsistent with the hypothesis that Hawai'i is a dead end for rare migrants from the Indo-Pacific. Instead, the weight of the evidence shows that these peripheral populations are not sinks, but important centers for the generation of genetic diversity feeding back towards the West Pacific. Specifically for *H. atra*, our data suggest that the pathway between Hawai'i and the rest of the Pacific is primarily out through Kingman Reef and the Line Islands and not in through Japan, the Marshall Islands, or the closest neighbor to the Hawaiian Archipelago, Johnston Atoll. We show that, at least for *H. atra*, Johnston Atoll is in fact an outpost of the Northwestern Hawaiian Islands and not a primary gateway for colonization of the Archipelago.

Considerable evidence is accumulating that it is indefensible to make predictions of connectivity based solely on proxies such as ecological or phylogenetic similarity, pelagic larval duration, or species range sizes [21, 23–25, 90]. The fine-scale structuring of populations in *H. atra* suggests that place-based management approaches, as exemplified by ecosystem-based management, are ideal for responding to the complex relationships between genetically distinct populations. *Holothuria atra* must be managed on a local scale; migration between archipelagos, and often between islands, does not occur in ecologically relevant time frames.

Appendices

A.

Phylogenetic tree of sampled *Holothuria atra* haplotypes and *Holothuria* and *Actinopyga* haplotypes retrieved from GenBank. The maximum likelihood tree is shown; Bayesian analysis produced a tree with almost identical topology. The first of the numbers beside branches corresponds to bootstrap support calculated from the maximum likelihood analysis; the second number, seperated by a /, corresponds to the posterior probability estimated during the Bayesian analysis. Only support values with 50% majority rule or 0.50 posterior probability are included. *Actinopyga agassizi* is the outgroup. All non-*H. atra* nodes were collapsed. GenBank accession numbers are included for uncollapsed taxa (see Figure 3).

B.

M and θ posterior probability distributions were calculated by MIGRATE using a Bayesian MCMC simulation. Population key: 1 = Kingman Reef; 2 = Line Islands; 3 = Main Hawaiian Islands; 4 = Northwestern Hawaiian Islands and Johnston Atoll; 5 = Okinawa; 6 = Ogasawara; 7 = Marshall Islands (see Table 5).

Table 5

Parameter	2.50%	25.00%	Mode	75.00%	97.50%	Median	Mean
$\theta 1$	0	0.0015	0.00375	0.0055	0.0115	0.00525	0.00518
$\theta 2$	0	0	0.00025	0.0015	0.0035	0.00175	0.00114
$\theta 3$	0	0.0015	0.00325	0.0045	0.007	0.00375	0.00345
$\theta 4$	0.001	0.0035	0.00525	0.007	0.0105	0.00625	0.00586
$\theta 5$	0	0	0.00025	0.0015	0.003	0.00175	0.00095
$\theta 6$	0	0	0.00025	0.001	0.003	0.00125	0.00085
$\theta 7$	0	0.0005	0.00225	0.0045	0.011	0.00375	0.00417
M2–>1	0	0	185	350	1150	345	426.5
M3–>1	0	90	225	560	1430	495	580.3
M4–>1	0	80	215	580	1460	525	609.3
M5–>1	0	110	235	530	1310	455	536.8
M6–>1	0	0	5	160	580	165	204.9
M7–>1	0	10	195	350	1110	335	417.3
M1–>2	0	0	125	260	1010	265	347.2
M3–>2	0	0	175	280	1000	285	363.1
M4–>2	0	0	45	220	860	225	293.6
M5–>2	0	20	205	490	1500	475	575.1
M6–>2	0	70	205	580	1500	525	612.6
M7–>2	0	0	195	360	1350	365	481.7
M1–>3	10	140	315	580	1490	515	609.7
M2–>3	0	0	5	160	620	165	208.8
M4–>3	0	150	295	660	1490	565	644.1
M5–>3	0	0	5	100	370	105	126.2
M6–>3	0	10	85	210	600	195	227.9
M7–>3	0	0	95	220	920	225	299.9
M1–>4	0	0	95	220	910	225	299.7
M2–>4	0	0	5	100	350	105	123.3
M3–>4	350	640	945	1340	1950	1085	1094.5
M5–>4	0	0	5	70	280	75	93.9
M6–>4	0	0	5	80	280	85	96.6
M7–>4	0	0	5	140	470	145	170.4
M1–>5	0	0	165	280	1050	285	371.1
M2–>5	0	0	185	350	1290	355	457.5
M3–>5	0	60	205	440	1260	385	480.6
M4–>5	0	0	115	260	960	265	344
M6–>5	0	0	175	300	1200	305	412.1
M7–>5	0	20	195	380	1200	355	444.4
M1–>6	0	0	115	270	1020	275	358
M2–>6	0	0	175	340	1240	345	440.7
M3–>6	0	0	105	250	910	255	322.7
M4–>6	0	0	125	250	950	255	330.6
M5–>6	0	0	175	310	1020	305	377.4
M7–>6	0	20	195	370	1170	345	434.6
M1–>7	0	0	185	320	1040	315	387.3
M2–>7	0	30	205	570	1580	535	637.2
M3–>7	0	40	195	440	1280	405	496.2
M4–>7	0	0	185	300	1040	305	385.1
M5–>7	0	0	205	580	1640	575	671.4
M6–>7	0	10	175	280	960	275	344

Table 6

Parameter	2.50%	25.00%	Mode	75.00%	97.50%	Median	Mean
$\theta 1$	0	0	0.0008	0.0015	0.0035	0.00175	0.00125
$\theta 2$	0.001	0.003	0.0048	0.0065	0.01	0.00575	0.00532
$\theta 3$	0.002	0.0045	0.0063	0.0085	0.012	0.00725	0.00709
$\theta 4$	0	0	0.0003	0.0015	0.003	0.00175	0.00099
$\theta 5$	0	0	0.0003	0.0015	0.004	0.00175	0.00127
$\theta 6$	0	0.0005	0.0023	0.004	0.0105	0.00375	0.00399
M2–>1	0	0	175	270	1000	275	356
M3–>1	0	0	5	210	840	215	286.9
M4–>1	0	10	205	530	1570	515	616.5
M5–>1	0	80	225	550	1410	485	573.5
M6–>1	0	0	185	340	1260	345	447.1
M1–>2	0	0	5	210	750	215	265.9
M3–>2	160	390	635	950	1700	805	858.6
M4–>2	0	0	5	120	420	125	150.1
M5–>2	0	0	5	80	310	85	106.6
M6–>2	0	0	95	230	790	235	294.7
M1–>3	0	0	5	80	320	85	108.3
M2–>3	210	460	695	1030	1750	885	926.5
M4–>3	0	0	5	60	210	65	70
M5–>3	0	0	5	100	290	105	111.2
M6–>3	0	0	5	100	360	105	123.9
M1–>4	0	0	185	390	1440	395	511.3
M2–>4	0	0	35	230	860	235	298.3
M3–>4	0	0	115	240	880	245	315.2
M5–>4	0	0	175	320	1170	325	417
M6–>4	0	0	175	330	1280	335	447.2
M1–>5	0	0	185	330	1190	325	422.7
M2–>5	0	0	55	230	930	235	316
M3–>5	0	0	5	190	700	195	243.8
M4–>5	0	40	195	380	1160	345	430.6
M6–>5	0	0	115	250	940	255	328.9
M1–>6	0	10	205	550	1640	535	650.1
M2–>6	0	120	245	570	1410	505	584.5
M3–>6	0	0	85	230	880	235	307.3
M4–>6	0	0	195	470	1510	475	581
M5–>6	0	0	175	270	940	275	345.8

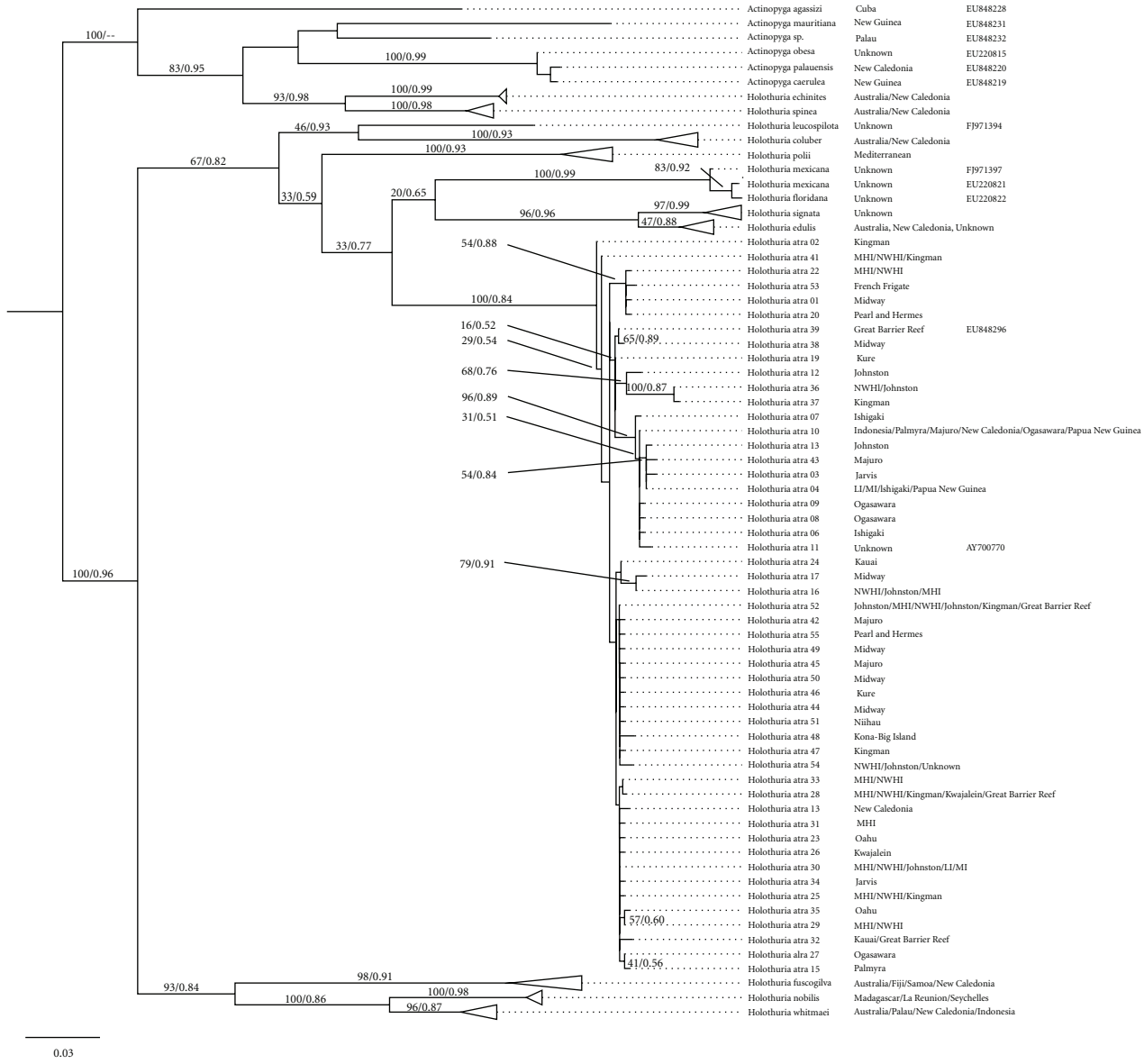

Figure 3

C.

M and θ posterior probability distributions were calculated by MIGRATE using a Bayesian MCMC simulation. Population key: 1 = Line Islands; 2 = Main Hawaiian Islands + Kingman; 3 = Northwestern Hawaiian Islands + Johnston; 4 = Okinawa; 5 = Ogasawara; 6 = Marshall Islands (see Table 6).

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