Final Report

Phylogeography of Giant Red Sea Cucumbers *Apostichopus californicus* in the Northeastern Pacific

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Abstract: The goal of this project was to provide information to harvest managers on the genetic population structure of giant sea cucumbers *Apostichopus californicus* in Alaska. A total of 17 samples (mean mtDNA N = 83.7; microsatellite N = 83.4) were collected extending from Kodiak Island, Alaska to Vancouver Island, British Columbia and examined with mitochondrial (mt) DNA cytochrome oxidase I (659 bp) and four microsatellite markers. The analysis of mtDNA haplotypes in 1423 individuals showed that haplotype diversity was large (h = 0.879) overall. A significant excess of low-frequency haplotypes appeared in each of the 17 samples. This departure from neutrality may indicate a recent population expansion. Only weak overall differentiation was detected among populations in Alaska (F_{ST} = 0.0023, P = 0.077), which was due to differences between three samples from Kodiak Island and 10 samples from southeastern Alaska. No significant difference appeared between a sample of hatchery juveniles and donor populations in southeastern Alaska (F_{ST} =0.0012, P = 0.606), nor was there a drop in genetic diversity in the hatchery sample. No significant difference appeared between the grouped Alaskan populations and three populations in British Columbia. Null alleles appeared in each of the four microsatellite loci and produced a strong difference between observed (H_O = 0.485) and expected (H_E= 0.824) heterozygosity. This heterozygote deficit led to a large inbreeding coefficient in each of the samples (mean: F_{IS} = 0.387). Measures of differentiation between populations were less influenced by the presence of null alleles. Pairwise divergence between samples ranged from F_{ST} = 0.0 to 0.033 for unadjusted allelic frequencies and from F_{ST} = 0.0 to 0.018 for adjusted allelic frequencies. Significant overall differentiation was detected among Alaskan populations (F_{ST} = 0.008, P <0.0001), and this was due to differences between populations around Kodiak Island and those in southeastern Alaska (F_{ST} = 0.0064, P = 0.003). No significant difference was found between hatchery juveniles and source populations (F_{ST} = 0.0026, P =0.194). The degree of heterogeneity between populations in Alaska and British Columbia was no greater than the heterogeneity within these two regions (F_{ST} = 0.0, P = 0.784). Significant isolation by distance appeared for microsatellite markers (r = 0.46, P = 0.0002), but not for mtDNA markers (r = 0.131, P = 0.194). Overall, these results indicate that populations of red sea cucumbers in Alaska can be managed on a regional scale. However, the protection of local populations is important for the maintenance of genetic diversity to support sustained yields.

Introduction

A key requirement for both the management of fisheries and development of stock enhancements is an understanding of how genetic diversity is distributed within and among populations (Ward 2006). Molecular genetic markers, together with ecological and biological data, can provide valuable information for the development of stock harvest and enhancement protocols (Thorpe et al. 2000). The assessment of genetic population structure is essential for defining stock boundaries for stock assessments and for selecting brood stock for hatchery culture. Genetic markers can also be used to monitor the effects of hatchery practices on genetic diversity in culture juveniles for use in aquaculture to stock supplementation (Bartley et al. 1992; Evans et al. 2004a), and to monitor the genetic effects cultured individuals released into the wild (Gaffney et al. 1996). Most notably, molecular markers can help to define independent demographic units to promote sustainable harvests (Begg et al. 1999). Stock definitions are needed, because small, but demographically independent, populations may be depressed if they are included with larger more abundant populations in the setting of harvest quotas.

This study focuses on the giant red sea cucumber *Apostichopus californicus* (Stimpson, 1857), which supports commercial dive fisheries in the Northeastern Pacific, largely for an Asian market. In Alaska, harvests have grown from about 1.0 million pounds in 1998–1999, increasing to 1.5 million pounds in 2013–2014. As this fishery has grown, stock abundances have noticeably declined in some areas (Hebert 2014). The growth of markets in North America and Asia have stimulated interest in supplementing wild production with hatchery-reared individuals to meet the demands of these markets (Palzat et al. 2008; Hannah et al. 2013).

Red sea cucumbers inhabit shallow subtidal areas along the Northeastern Pacific coast from Baja California to Alaska. This sea cucumber is one of several species in the genus *Apostichopus* that are distributed throughout the Pacific Ocean (Arndt et al. 1996). It has a has large, soft, cylindrical body that can reach 50 cm in length. This sea cucumber is covered with red-brown to yellowish leathery skin with nodules. It is a scavenger and feeds by sifting through sediments with tentacles, or by catching plankton from currents with 20 tentacles around the mouth (Camaron and Frankboner 1984). Five rows of tube feet extend along the body and provide a limited amount of movement, largely at night. When disturbed, or in contact with sea-star

predators, individuals flex their bodies rapidly dislodging themselves from the bottom and moving some distance in the current (Margolin 1976; Da Silva et al. 1986). Sexes are separate and spawn with external fertilization generally takes place in Spring and Summer. Planktonic larvae spend a few weeks drifting in the water, then metamorphose into the adult form and settle to the bottom (Camaron and Fankboner 1986). In culture, larvae develop at different rates, indicating asynchronous metamorphosis and settlement among a seasonal cohort occurs over several months. When food is limiting, individuals metabolize body mass to survive (Camaron and Fankboner 1989). In Alaska, individuals can be found in some abundance to depths of 180 m (Woodby et al. 2000; Zhou and Shirley 1996).

Exploited wild populations can be viewed in two ways (Andrewartha and Birch 1984). First, a species can be viewed as consisting of *ecological* populations, which are groups of interacting individuals that are affected by density dependent processes. This definition of population is the primary focus of fishery management. Second, the *evolutionary* population shows genetic continuity over time and is often the focus of conservation concerns about continued persistence. The ecological perspective of a population in fishery management is short, reaching back at most decades, or often only from year to year, whereas the evolutionary perspective of a population extends over millennia and longer. Both short-term ecological processes and long-term environmental processes influence the genetic variability seen in present-day populations and the patterns of variability from both processes must be considered in the use of genetic data for fishery management.

The focus of this project is on the genetic structure of red sea cucumbers to aid in the management of harvested populations (ADF&G 2009). Both contemporary ecologically driven genetic processes, such as random genetic drift and gene flow between populations and evolutionary events, such as range expansions and founder events can be assessed with complementary molecular methods (Grant and Bowen 1998). Near-shore habitats in the Northeastern Pacific are influenced by climate cycles on several temporal scales. On decadal time scales, the Pacific Decadal Oscillation (PDO) can have a substantial influence on the abundances and distributions of shallow-water marine species (Mantua and Hare 2002). For example, the Gulf of Alaska transitioned from a crustacean-dominated ecosystem to a fish-dominated ecosystem during 1977–1978 the PDO climate shift (Anderson and Piatt 1999;

Litzow and Ciannelli 2007). These climatic changes may lead to range shifts and to population declines or extirpations of local populations, which influence patterns of genetic diversity among populations.

On longer temporal scales of tens of thousands of years, shorelines around the Northeastern Pacific were covered with tidewater glaciers that were an extension of massive continental glaciers. During the Last Glacial Maximum 20–18 thousand years ago (kya), global sea levels dropped about 100 meters and terrestrial glacier margins blanketed the Northeastern Pacific shorelines, except for a few refugial areas, including Kodiak and Queen Charlotte islands (Karlstrom and Ball 1969; Mann and Hamilton 1995). The survival of populations in multiple refugia often leads to deep genetic divergences between regional populations as seen in Pacific cod *Gadus microcephalus* (Canino et al. 2010) and red king crabs *Paralithodes camtschaticus* (Grant and Cheng 2012). Post-glacial population expansions into new habitats from a single, or a few, population(s) may lead to shallow population structure and the accumulation of low-frequency mutations (Hewitt 2004).

While long-term environmental changes may be responsible for deep levels of genetic structure, on-going levels of connectivity between populations are of more immediate interest to fishery managers who attempt to manage harvested populations on annual or decadal time scales. Estimates of gene flow from patterns of allele-frequency variability can be used to infer the extent of migration and connectivity between populations (Waples and Gaggiotti 2006). Levels of variability in various classes of DNA in the genome are influenced by modes of inheritance and mutation rates, and the analysis of these various markers can provide complementary views of genetic structure. Maternally inherited mtDNA, for example, can be used to trace deep genetic lineages because mtDNA genes are passed on from one generation to the next without recombination. Microsatellite DNA, on the other hand, may more useful for estimating the genetic effects of contemporary events because of a large mutation rate (Karl et al. 2012.

The goal of this study was to survey genetic variability among red sea cucumbers with mtDNA and microsatellite DNA markers to resolve genetic population structure over a broad geographical scale. While molecular markers give insight into the demographic features of population structure, they may not detect adaptive differences among populations. In many

marine species, the spatial and temporal scales of adaptive variation are often smaller than the scales of genetic differentiation detected with neutral molecular markers (Conover et al. 2006). In most cases, adaptive variation can only be detected with laboratory or common garden experiments, which may be difficult to design and expensive to conduct (Evans et al. 2004b). Nevertheless, the maintenance of adaptive variability on an appropriate spatial scale is essential to protect the genetic integrity of wild populations. The use of neutral genetic markers is the first step toward identifying an adaptive seascape for red sea cucumbers.

Materials and Methods

Sample collection and DNA extraction

Samples from 16 localities in the Northeastern Pacific and one sample of offspring from a hatchery (Alutiiq Pride Shellfish Hatchery, Seward, Alaska) were examined for molecular variability (Figure 1). Muscle was dissected from sea cucumbers collected during commercial dive operations or from specimens collected during research surveys by the Alaska Department of Fish and Game. DNA extracts from three samples of sea cucumbers collected in British Columbia were furnished by John Nelson. Samples were preserved in 95% ethanol until DNA extraction. DNA was extracted from tissues with NucleoSpin® 96 Tissue (Machery-Nagel Inc., Bethlehem, PA).

Mitochondrial DNA sequences

An edited 659 base pair (bp) fragment of cytochrome oxidase subunit I (COI) was amplified with the polymerase chain reaction (PCR) using the general echinoderm primers COIe-F—5’-ATAATGATAGGAGGRTTTGG-3’ and COIe-R—5’-GCTCGTGTRTCTACRTCCAT-3’ (Jacobs et al. 1988; Arndt et al. 1996; So et al. 2011) PCR cocktails consisted of a 50 µL mixture of 2.0 µL templates DNA in 1x Colorless GoTaq Flexi Buffer, 2.5 mM $MgCl_2$, 0.8 mM of each dNTP, 1 µM of forward and reverse primers, and 2.5U GoTaq Flexi DNA polymerase. PCR amplification were conducted in ABI 9700 thermocyclers with an initial denaturation of 1 min at 95^{o}C, 32 cycles of 30 s at 95^{o}C, 30 s at primer annealing temperature 50 ^{o}C, and 1 min 20 s at 72^{o}C; the final cycle was at 72^{o}C for 10 min. The PCR amplifications were sequenced in the forward and reverse directions by Genewiz Inc. (South Plainfield, NJ). Forward and reverse-

complement sequences were aligned and edited with MEGA 7.0.20 (Kumar et al. 2016) and Finch TV 1.4.0 (Geospiza Inc.) to produce a 659 bp fragment for population analyses.

Microsatellite genotyping

We used a suite of 4 microsatellite loci (*Psc1, Psc2, Psc4,* and *Psc5*) that had previously been developed for red sea cucumbers (Nelson et al. 2002) (Table 1). Polymerase chain reaction was used to amplify microsatellite alleles with a Gene Amp PCR System 9700 (Applied Biosystems, Inc., Foster City, CA). Each 10 µL reaction mixture consisted of 2 µL template DNA (~0.1µg/µL) in 1x Colorless GoTaq Flexi Buffer (Promega Inc. Madison, WI), 2 mM $MgCl_2$ (Promega Inc. Madison, WI), 0.20 mM of each nucleotide (Applied Biogsytems, Inc.), 0.4 µM of forward and reverse primers, 0.1 mg/mL of BSA (Sigma Inc. St. Louis, MO), 0.05 U GoTaq Flexi DNA polymerase (Promega Inc. Madison, WI), and deionized water. Optimal thermal cycling profiles varied among loci (Table S1). Microsatellites were size fractionated in an Applied Biosystems 3730 capillary DNA sequencer. Genotypes were scored with GeneMapper 5.0 (Applied Biosystems) independently by two technicians. A subset of 8% of the samples was re-extracted and re-genotyped by a third technician to make sure samples had not been mixed in some way.

Statistical analyses: mtDNA

Mitochondrial DNA sequences were used to resolve various features of genetic structure in red sea cucumbers. A 95% plausible parsimony network of mtDNA haplotypes was constructed with TCS 1.21 (Clement et al. 2000) to show relative frequencies and relationships among haplotypes. We used ARLEQUIN 3.5.2.2 (Excoffier and Lischer 2010) to estimate the number of polymorphic nucleotide sites, N_{Poly}, the number of nucleotide transitions, *ts*, and transversions, *tv*, the number of private haplotypes, N_{Priv}, the number of observed, N_h, and expected, N_{Exp}, haplotypes, gene diversity, *h*, and nucleotide diversity, θ_π.

We analyzed mtDNA haplotypic and microsatellite genotypic frequencies to elicit features of genetic population structure. The power to detect genetic differences with the sample sizes and number of markers used in this study was estimated with the simulation program of Ryman and Palm (2006) by assuming an effective population size of 2000 individuals (Table S2). The extent

of divergence between populations was estimated with *F* statistics (Weir and Cockerham 1984) in ARLEQUIN. A neighbor-joining tree was constructed from the matrix of pairwise values of F_{ST} with MEGA7 to show the genetic relationships among samples. Isolation by distance (IBD) was measured with the software package IBDWS (*http://ibdws.sdsu.edu/~ibdws/*; Jensen et al. 2005) to estimate Mantel's correlation coefficient between genetic and geographic distance. Significance was determined with10,000 randomizations to test the one-sided null hypotheses that the correlation was greater than or equal to 0.0. Tests were made with geographic distances in kilometers and with the log of the geographic distances between samples to resolve IBD on different geographic scales. One test for IBD was made with geographic distances in kilometers and a second with the log of the geographic distances to resolve possible IBD on different geographic scales.

We used the analysis of molecular variation (AMOVA) in ARLEQUIN to explore geographical structure in the distributions of mtDNA-haplotype frequencies. AMOVAs of haplotype-frequency variability between samples, as measured with F_{ST}, were used to provide insights into the degree of genetic connectivity between various population groups. Groupings were based on geography.

Microsatellite DNA

We made an initial analysis of genotype data with GENEPOP 4.6 (Rousset 2007) and found evidence that null alleles were pervasive in *Pca_Psc1*, *Pca_Psc2*, and *Pca_Psc4*, but not *Pca_Psc5*. The analysis with MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) confirmed the presence of null alleles, but found little evidence of stuttering and large-allele dropout. Some loci failed to amplify with PCR in some individuals, even though amplifications were successful at other loci. These PCR failures were assumed to be null-allele homozygotes.

We used several programs to estimate null-allele frequencies. Before adjusting for null alleles, we took out individuals for which no PCR products appeared for any of the four loci, assuming these failures were due to poor sample quality and not to null alleles. We then estimated null-allele frequencies with the programs MICRO-CHECKER, ML-NULLFREQ (Kalinowski and Taper 2006), and FreeNA (Chapuis and Estoup 2007). MICRO-CHECKER produces estimates of null-allele frequencies with algorithms derived by Van Oosterhout et al.

(2004), Charkraborty et al. (1997), and Brookfield (1996). ML-NULLFREQ produces maximum likelihood estimates of null- and adjusted-allele frequencies with the iterative Expectation Maximization (EM) algorithm of Dempster et al. (1977). Starting values in the latter program are estimated by the null allele estimator of Chakraborty et al. (1992) from observed and expected heterozygosities under Hardy Weinberg equilibrium. ML-NULLFREQ also estimates the probability, β, that apparent genotyping failures resulted from something other than the homozygosity of null alleles. The maximum likelihood approach performs better than other estimators of null alleles, including those of Charkarborty et al. (1997) and Brookfield (1996) (Chapuis and Estoup 2007). FreeNA also estimates null- and adjusted- allele frequencies with maximum likelihood using EM algorithm, but with starting values based observed allele frequencies estimated with gene counting.

Genetic population structure

We examined the distributions of microsatellite genotypes within and among samples in several ways to resolve important features of population structure. We estimated F_{ST} (Weir and Cockerham 1984) from the original genotypes and from genotypes adjusted for null alleles with FreeNA. Neighbor-joining trees of F_{ST}, without and with corrections for null alleles (F_{ST}^{ENA}), were produced with MEGA 7. Isolation by distance was tested with Mantel's correlation between difference matrices of pairwise $F_{ST}/(1-F_{ST})$ and approximate shoreline distances between samples, as above.

We used the analysis of molecular variation (AMOVA) in ARLEQUIN to test for significant differences between populations. The same groupings as used for mtDNA were based on geography.

Results

Mitochondrial DNA

A total of 135 polymorphic nucleotide sites along a 659 base-pair segment of the mtDNA cytochrome oxidase I (COI) gene defined 170 haplotypes among 1423 sea cucumbers in 17 samples from the shores of the Northeastern Pacific Ocean extending from Kodiak Island, Alaska to Vancouver Island, British Columbia (Table 2, S3, S4 Figure 1). A haplotype network

of mutational relations among haplotypes showed an abundant central haplotype (1) in 30.2% of the individuals with numerous singleton haplotypes or low-frequency haplotypes that were 1–4 mutations removed from the central haplotype (Figure 2).

Sample sizes per location ranged from N = 54 to 95 and averaged 83.7 (Table 2). The number of polymorphic DNA sites varied from N_{poly} = 30 to 45 and averaged 38.2 among samples. The number of private haplotypes present in only one sample ranged from N_{Priv} = 0 to 9 and averaged 3.3 haplotypes per sample. The overall transition:transversion ratio along the COI segment was 4.8. The number of haplotypes per sample ranged from N_h = 21 to 37 and averaged 29.1. The expected numbers of haplotypes under neutrality were much smaller, ranging from N_{Exp} = 10.92 to 21.31 and averaged 17.12. Gene diversity, an analogue of diploid heterozygosity, ranged from h = 0.791 to 0.921 and averaged 0.877 among samples. Nucleotide diversity ranged from θ_π = 0.0027 to 0.0048 and averaged 0.0040 among samples.

Tests for departure from neutrality were significant in all of the samples (Table 2). D_T was similar among samples ranging from -1.86 to -2.44 and -2.36 overall. All of these values were significantly less than 0.0 ($P < 0.038$). F_S ranged from -9.85 to -27.19 and -25.30 overall ($P < 0.0015$). These significant departures reflect an excess of low-frequency haplotypes that are apparent in the haplotype network (Figure 1) and in the observed and expected numbers of haplotypes (Table 2).

Genetic population structure was tested with several statistics. Divergence between populations was estimated with F_{ST} between pairs of samples (Table 3). A total of 54 pairwise F_{ST} values were negative or equal to 0.0. Thirteen of the 136 comparisons were significant with P = 0.05, but only 11 (8.1%) were significant after a Bonferroni correction for multiple comparisons. Among these, samples 12 and 17 each showed five significant comparisons with other samples. A neighbor-joining tree of F_{ST} showed only weak geographic trends in the genetic relationships among samples (Figure 3a). Samples 12 and 17, which had divergent values of F_{ST}, did not appear to be outliers from the other samples. Mantel's test showed a weak positive, but not significant, correlation (r = 0.131, P = 0.194) between genetic [$F_{ST}/(1 - F_{ST})$] and geographical [log(km)] distances between samples (Figure 4a).

Several AMOVAs were made with various partitions to resolve possible population structure indicated by the significant pairwise values of F_{ST} (Table 4). An overall comparison of the samples from Alaska was not significant (F_{ST} = 0.002, P = 0.077), nor were any of the comparisons of samples within Alaska, except for significant heterogeneity among samples 10–13 in the southern part of southeastern Alaska. Notably, a comparison of the one hatchery sample with populations in the brood stock source area was not significant (F_{ST} = 0.0012, P = 0.606). A comparison between the Alaskan samples (N = 13) and those from British Columbia (N = 3) was also not significant, but significant heterogeneity appeared between the three samples from British Columbia.

Microsatellite DNA

We used four previously developed microsatellite loci to help resolve the genetic population structure of red sea cucumbers (Nelson et al. 2002; Table S5). The number of alleles per locus ranged from A_N = 12 (*Pca_Psc5*) to 38 (*Pca_Psc1*) (Table 5). Observed heterozygosities ranged from H_O = 0.340 (*Pca_Psc2*) to 0.666 (*Pca_Psc1*) (mean = 0.485) and were considerably smaller than expected heterozygosities, which ranged from H_E = 0.643 (*Pca_Psc5*) to 0.948 (*Pca_Psc1*) (mean: 0.824). GenePop and MICRO-CHECKER indicated that this contrast in diversity was due to the presence of null alleles. We then used ML-NULLFREQ to estimate null-allele frequencies (Table 5, S5) and FreeNa to estimate F_{ST} adjusted for null alleles. The contrast between adjusted (F_{ST}-ENA) and unadjusted estimates of F_{ST} was not great. The mean unadjusted F_{ST} was 0.0041 (range: 0.0004–0.0098), and mean adjusted F_{ST}-ENA was 0.0037 (range: 0.0002–0.0087).

Patterns of diversity among samples were estimated in several ways. A total of 107 alleles were observed among samples, but the number of alleles per sample was considerably smaller, ranging from A_N = 15.0 to 19.8 (mean: 17.6) (Table 6). Allelic richness, based on a sample size of 52, ranged from A_R = 14.8 to 17.2 (mean: 16.0) and did not show a geographical gradient. The number of private alleles occurring in only one sample ranged from A_{PRI} = 0.0 to 0.55 (mean: 0.19). The three samples from British Columbia had the largest numbers of private alleles (range: 0.40–0.55). Observed heterozygosity ranged from H_O = 0.438 to 0.521 (mean: 0.484) and was much less than expected heterozygosity, which ranged from H_E = 0.774 to 0.820 (mean: 0.802).

This difference is due the presence of null alleles. Estimates of inbreeding unadjusted for null alleles were large, ranging from F_{IS} = 0.341 to 0.443 and from Rho_{ST} = 0.051 to 0.559. The three southernmost samples (14–16) from British Columbia had larger values of Rho_{ST} (range: 0.427–0.456) than did samples from Alaska (range: 0.051–0.385), except for the Alaskan hatchery samples (0.559).

Unadjusted and adjusted (for null alleles) estimates of F_{ST} were similar; F_{ST} between samples ranged from 0.0 (17 negative values) to 0.0333 (sample 6 vs 17-hatchery) between samples, whereas F_{ST}^{ENA} ranged from 0.0 to 0.0189 (sample 1 vs 17) (Table 7). A total of 39 comparison were significant at P = 0.05, but only 3 were significant after Bonferroni correction of P-values among 136 pairwise comparisons. Neighbor-joining trees for F_{ST} and F_{ST}^{ENA} showed only weak geographic trends in the relationships among populations. Mantel's test for isolation by distance showed a significant positive relationship between F_{ST} and geographical distance (r = 0.455, P = 0.0002), as did F_{ST}^{ENA} (r = 0.426, P = 0.0005) (Figure 4).

An AMOVA of the 13 Alaskan samples showed a significant amount of overall heterogeneity (F_{ST} = 0.0076, P < 0.0001) (Table 8). Several partitions were made to illicit the sources of this heterogeneity. A comparison of the samples from Kodiak Island (samples 1–3) with the rest of the samples from Alaska (samples 4–13) indicated significant differences between these two groups (F_{ST} = 0.0064, P = 0.003) and among populations within these groups (F_{SC} = 0.0053, P = 0.001). A four-way comparison between samples from Kodiak Island (samples 1–3), Yakutat (sample 4), northern southeastern Alaska (samples 5–9), and southern southeastern Alaska (samples 10–13) was highly significant between groups (F_{ST} = 0.0047, P <0.0001), but not within groups on average (F_{SC} = 0.0041, P = 0.060). No significant heterogeneity appeared within the samples from southeastern Alaska. The sample of hatchery juveniles was not significantly diverged from brood-stock donor populations in southern southeastern Alaska. A comparison between samples from Alaska (samples 1–13) and three samples from British Columbia (samples 14-16) was not significant (F_{ST} = 0.00001, P = 0.784), nor was a comparison between the three samples within British Columbia (F_{ST} = 0.0033, P = 0.293)

Discussion

While both ecological and genetic information can be used to define management units, we focused here on the use of genetic markers to delineate genetically distinctive population groups of red sea cucumbers in the Northeastern Pacific Ocean. Our survey of mtDNA haplotype and microsatellite allele-frequency variability resolved genetic structure on only regional scales, and hatchery juveniles were not divergent from donor populations. Before discussing the results of this study in more detail, we provide an evaluation of the ability of mtDNA and microsatellite markers to detect genetic variability among populations of an apparently high gene-flow species.

First, for a single locus, such as mtDNA, samples sizes of about 60 to 90 provide power of only about 10% for detecting differences on the order of F_{ST} = 0.001, but power of about 70% for populations showing higher levels of differentiation F_{ST} = 0.010 (Table S2). However, statistical power is 100% for even small values of F_{ST} when samples are pooled for comparisons between regions. While the statistical power for detecting differences with mtDNA is low, this marker provides phylogeographic information not possible with nuclear makers. For four microsatellite loci, the power of detecting an F_{ST} = 0.001 is about 25%, but reaches 90% for F_{ST} = 0.010. When samples are pooled for regional comparisons, these samples sizes and number of loci provide statistical power of 100% for detecting differences at even low levels of divergence. Given our sample sizes and number of markers, we are confident that the sample design used in this study provides adequate resolution of genetic population structure that would be useful for the management of sea cucumber populations.

Second, three of the microsatellite loci that we used in this study had null alleles. The inability to detect heterozygotes for the null alleles produced an excess of apparent homozygous genotypes. Null alleles at microsatellite loci appear to be common in marine invertebrates (Chiesa et al. 2016; Hargrove et al. 2015; Rico et al. 2017) and appear to be due to mutations in PCR primer sites that lead to the failure of PCR amplification by one or both of the primers. In our study, null alleles led to a strong contrast between estimates of observed and expected heterozygosity. Observed heterozygosity is based on counts of heterozygous genotypes, some of which cannot be identified, while expected heterozygosity is calculated from allele frequencies. Hence, estimates of observed diversity can be used to make relative comparisons among populations, but only expected heterozygosity is appropriate for making comparisons with other species. Estimates of divergence between populations are also calculated from allelic

frequencies, and when null alleles are more or less evenly distributed among samples, which for the most part they were (Table S6), estimates of F_{ST} with and without adjustments for nulls alleles are expected to be similar (Table 7). Hence, we are confident that the patterns of divergence estimated with F_{ST} are reliable for making inferences about genetic population structure.

A third consideration is the interpretation of genetic data. Estimates of stock structure from genetic markers have weaknesses that need to be considered in their application to fishery problems. Estimates of migration from genetic data (e.g. F_{ST}) are generally not useful to managers because these estimates assume an equilibrium between random drift and migration, and because estimates consist of the number of migrants (N_m) and not the migration rate (m). Managers are interested in stock structure estimates based on contemporary processes and in the proportion of individuals migrating between stocks that influence demographic independence. When a significant genetic difference is detected between populations in two areas, they are also likely to be demographically different, perhaps with different maturity schedules, different fecundities, or different age class structures, depending on environmental differences between the areas. However, the lack of genetic distinction between populations does not preclude ecological differences that could be used to manage the populations separately. Simulations indicate that populations with migration rates as large as 10% can still be demographically independent of one another (Waples and Gaggiotti 2006). This large rate of migration would produce genetic homogeneity, so that the demographic independence between stocks would not be detectable with genetic markers.

Using both unadjusted and adjusted allele frequencies, we found little genetic structure among populations on geographical scales of 100 km. Individuals along the coast of British Columbia spawn at 4–8 years of age and live to about 12 years of age (Cameron and Fankboner 1989). Some movement may occur during feeding or when individuals dislodge from the bottom in the presence of predators. Some gene flow may occur when gametes are spawned externally. However, the lack of structure is most likely attributable to the dispersal of planktonic larvae, which provides a link between populations. Dispersal of larvae in coastal currents for 2–4 months before metamorphosing and settling to the bottom is likely the most important mechanisms of gene flow between populations. Nevertheless, high levels of predation and larval

retention eddies may limit along-shore movement and lead to various patterns of recruitment and gene flow in different regions.

The general lack of genetic population structure that we found in Alaska differs somewhat from the population structure in British Columbia. In a study of microsatellite variability among populations in British Columbia, Nelson and Cooper (2003) found a chaotic genetic population structure. Populations that were more or less isolated in some inlets were genetically homogeneous, whereas in other areas, populations appeared to be isolated by local current eddies, producing genetic heterogeneity on the same spatial scale. While no clear pattern of isolation by distance appeared overall among samples extending over about 1300 km, isolation by distance and genetic differences appeared among populations on scales of tens to hundreds of km. These results show that, despite a lengthy pelagic larval stage, genetically distinctive populations of red sea cucumbers can arise because of isolation in bays or because of isolation by nearshore currents.

Genetic markers in sea cucumber populations in other areas have revealed considerable genetic population structure. A study of the sea cucumber *Holothuria scabra* in South Pacific found genetic differences not only on large regional scales (New Caledonia, Australia, Solomon Islands), but also between populations in embayments separated by tens of kilometers (Uthicke and Purcell 2004). The analysis of microsatellite markers also demonstrated genetic differences among populations of *Stichopus japonicus* in the Yellow Sea and the northern part of the Sea of Japan (Kim et al. 2008; Chang et al. 2009). Microsatellite markers also showed that a red color morph of *Stichopus japonicus* was genetically differentiated from green and black morphs, and that these morphs likely represented distinct species (Kanno et al. 2006).

An important application of genetic data is to the development of hatchery stocks to supplement wild populations (Grant et al. 2017). In Alaska, harvests and predation from sea otters have led to declines red sea cucumbers in some areas, some of which have been closed to harvesting (McManman 2009; Hebert 2014). As a result, harvesters would like to initiate a population-enhancement program in which hatchery-reared individuals would be released into the wild (Decker 2009). Experiments have been performed by the Alutiiq Pride Shellfish Hatchery (APSH) and the Southeast Alaska Regional Diver Fisheries Association (SARDFA) to

evaluate whether sea cucumber culture is possible (McManman, 2009). Our sample of hatchery juveniles from did not show a drop in genetic diversity, nor significant divergence from donor populations. This indicates that brood-stock sizes were large enough and culture practices sufficient to retain adequate stores of genetic diversity.

Genetic population structure can be partitioned into the effects of ongoing processes, such as genetic drift and gene flow, and the effects of historical events. Genetic drift and gene flow are expected to be the chief drivers of genetic population structure in equilibrium populations and the effects of these processes can be detected with haplotype-frequency analysis of mtDNA and allele-frequency analysis of microsatellites. Population structure at this level is often most useful for harvest managers. However, deeper structure producing evolutionarily distinctive population groups must also be considered. On long time scales, periodic coastal glaciations along the shores of the Northeastern Pacific led to population isolations, extinctions, dispersals, and founder effects that produced deep genetic imprints. The analysis of mtDNA can often be used to detect these imprints. For example, Pacific herring *Clupea pallasi* (Grant and Utter 1984; Wildes et al. 2011), Pacific cod *Gadus macrocephalus* (Canino et al. 2010), and red king crab *Paralithodes camtschaticus* (Grant and Cheng 2012; Vulstek et al. 2013) show deep genetic partitions across the North Pacific that are legacies of Pleistocene isolations.

While we found no evidence of deep genetic partitions among populations of red sea cucumbers in the Northeastern Pacific Ocean that could be attributed to divergence in isolation during ice age maxima, we found strong departures from neutrality indicating that the populations are not in evolutionary equilibrium. One explanation for this departure invokes regional contractions of populations into refugia and post-glacial expansions into newly available habitats. Recent population expansions typically produce a skewed allele-frequency spectrum with an excess of low-frequency mutations, which are retained to a greater degree in expanding populations than they are in stable or contracting populations.

In conclusion, this study shows several general results. First, the ragged Alaska coastline and current gyres in south-central and southeastern Alaska do not appear to isolate local populations of sea cucumbers sufficiently to produce genetic differences among them. Hence, regional populations could potentially be treated as a single management unit, from at least a genetic’s

perspective. Second, hatchery practices appear to be adequate to prevent divergence from donor populations and to maintain genetic diversity in hatchery-reared juveniles. Another finding is that, even though sea cucumber populations are at the edge of the species' range, they do not show a drop in genetic diversity that has been observed for populations at the edge of a rang in some species (Hampe and Petit 2005; McInerny et al. 2009). Lastly, our survey revealed genetic imprints that likely emanate from events in the late Pleistocene.

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Figure captions

Figure 1. Map showing locations of samples of giant red sea cucumber *Apostichopus californicus* in the Northeastern Pacific Ocean,

Figure 2. Haplotype network showing mutational relationships among mitochondrial DNA cytochrome oxidase I (*base pairs* = 659) haplotypes of giant red sea cucumber *Apostichopus californicus* in the Northeastern Pacific Ocean. Lines connecting haplotypes (open circles), or hypothesized haplotypes (closed circles), represent a single mutation. Circles are proportional to haplotype frequency.

Figure 3. Neighbor-joining tree of F_{ST} between samples of giant red sea cucumber *Apostichopus californicus*. Sample numbers correspond to those in Table 1 and Figure 1. (a) F_{ST} estimated from mitochondrial DNA cytochrome oxidase I sequences (*base pairs* = 659). (b) F_{ST} estimated from the original microsatellite allelic frequencies. (b) F_{ST}^{ENA} estimated from the microsatellite allelic frequencies adjusted for null alleles using FreeNA.

Figure 4. Isolation by distance based on comparison between genetic and geographical distances between samples of giant red sea cucumber *Apostichopus californicus* extending from Kodiak Island, Alaska to Vancouver Island, British Columbia. Geographic distances between samples were log transformed. (a) mtDNA: $r = 0.131$, $P = 0.194$. (b) microsatellite F_{ST} unadjusted for null alleles: $r = 0.455$, $P = 0.0002$. (c) microsatellite F_{ST}^{ENA} adjusted for null alleles: $r = 0.426$, $P = 0.0005$.

Table 1. Microsatellite loci used to study the genetic population structure of giant red sea cucumbers *Apostichopus californicus* in the Northwestern Pacific Ocean. Information modified from Nelson et al. (2002).

Locus	Repetitive motif	Primer sequence (5' to 3')	Ta °C	GenBank Accession no.
Psc1	$(GACA)_4(GATA)_3(GACA)_4$TACAGA TAGACAGATAGACAGATA$(GACA)_4$	L-CACACGAAAACAAACTAGAACACAT R-TCGTTTGTTGCCCATTTGTA	47	AF455029
Psc2	$(ACGG)_2CGG(ACGG)_4$	L-TCTAGGCTAGCCAAACCAAAA R-GATCAAAATTGCATCCACCA	47	AF455030
Psc4	$(TTA)_6$	L-CTAGAGGATCGTAAGGGTTACACAGG R-TTCGTGTTCGCTGATGAAAAGT	54	AF455032
Psc5	(ATTTAGCTC)A$(ATTTAGCTC)_3$	L-ACCGCCCTACATCCTCTC R-TAGACTGGCATTAAAATTAGACAAAC	50	AF455033

Table 2. Sample information and summary statistics of mitochondrial DNA cytochrome oxidase I (659 bp) sequence variability in giant red sea cucumbers (*Apostichopus californicus*). *N* = sample size. N_{Poly} = number of polymorphic nucleotide sites. N_{Priv} = number of private alleles in sample. *ts* = number of transitions. *tv* = number of transversions. N_h = number of haplotypes. N_{Exp} = number of haplotypes expected under neutrality. *h* = gene diversity (based on haplotype frequencies). θ_π = nucleotide diversity (based on haplotype frequencies and number of substitutions between haplotypes). D_T = Tajima's measure of departure from neutrality. F_S = Fu's measure of departure from neutrality. All values of D_T were significant at $P = 0.038$ or less. All values of F_S were significant at $P = 0.0015$ or less.

				mtDNA									
Location	Date	Latitude	Longitude	*N*	N_{Poly}	N_{Priv}	*ts*	*tv*	N_h	N_{Exp}	*h*	θ_π %	D_T	F_S
1. Larsen Bay	2011	57.5270	-154.0727	93	45	9	40	6	31	12.99	0.828	0.310	-2.44	-27.19
2. Killiuda Bay	2011	57.3377	-152.9057	95	30	0	27	4	24	10.92	0.791	0.272	-2.12	-18.40
3. Kitoi Bay	2011	58.1884	-152.3632	58	32	1	29	4	23	18.30	0.911	0.453	-1.86	-13.19
4. Yakutat Bay	2010	59.7510	-139.6520	93	43	7	38	6	30	18.86	0.890	0.419	-2.13	-21.08
5. Barlow Cove	2010	58.3589	-134.9059	81	37	1	34	4	29	16.39	0.876	0.400	-2.06	-21.83
6. South Lynn Canal	2010	58.2725	-135.0977	95	42	4	38	6	37	19.29	0.892	0.423	-2.08	-26.89
7. West Chatham Bay	2010	57.9065	-135.0762	94	41	4	38	3	31	17.94	0.882	0.406	-2.10	-23.32
8. Windham Bay	2010	57.5845	-133.4816	94	44	3	40	5	35	18.16	0.884	0.453	-2.07	-26.35
9. North Sitka Sound	2010	57.2004	-135.4147	78	40	3	36	4	31	15.76	0.872	0.409	-2.15	-25.34
10. Sea Otter Sound	2011	56.0489	-133.2974	54	39	3	36	3	25	19.22	0.921	0.465	-2.16	-16.91
11. Zimovia Strait	2011	56.1868	-132.2390	92	40	2	35	5	32	21.31	0.907	0.451	-1.96	-23.14
12. Fillmore Inlet	2010	54.9178	-130.4745	93	40	4	37	3	33	19.82	0.897	0.480	-1.88	-23.37
13. Lower Cordova Bay	2010	54.8780	-132.3488	95	40	2	37	4	32	19.87	0.896	0.372	-2.16	-26.25
14. Bella Bella	2002	52.1520	-128.1386	61	30	4	27	3	22	12.28	0.843	0.292	-2.26	-17.35
15. Tofino Inlet	2001	49.1526	-125.8952	95	39	5	37	2	29	20.31	0.899	0.365	-2.15	-21.86
16. Jervis Inlet	2002	50.0547	-123.8199	83	29	2	25	5	21	15.50	0.866	0.381	-1.76	- 9.85
17. Alutiiq Pride Shellfish Hatchery	2002	–	–	69	38	2	34	4	29	14.12	0.861	0.411	-2.14	-23.29
Mean	–	–	–	83.7	38.2	3.3	34.6	4,2	29.1	17.12	0.877	0.398	-2.19	-21.51
Total	–	–	–	1423	135	–	120	25	170	17.03	0.879	0.398	-2.36	-25.30

Table 3. Pairwise values of F_{ST} between samples of giant red sea cucumbers from the Northeastern Pacific Ocean. Sample numbers as in Table 1 and Figure 1. F_{ST} values in italics and bold were significant at $P = 0.05$, but after Bonferroni correction for multiple tests only values in bold were significant.

	Sample number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	-0.0026															
3	0.0063	0.0008														
4	0.0019	-0.0005	-0.0007													
5	0.0025	0.0004	-0.0029	-0.0042												
6	0.0047	0.0003	-0.0033	-0.0033	-0.0081											
7	0.0038	0.0021	0.0014	-0.0021	-0.0084	-0.0049										
8	*0.0173*	0.0140	0.0018	0.0073	-0.0038	-0.0015	-0.0025									
9	0.0029	0.0007	-0.0003	-0.0034	-0.0041	-0.0041	-0.0029	0.0023								
10	0.0030	0.0025	-0.0074	-0.0035	-0.0074	-0.0085	-0.0055	-0.0045	-0.0081							
11	0.0078	0.0069	0.0016	-0.0035	-0.0071	-0.0047	-0.0046	-0.0024	-0.0026	-0.0065						
12	**0.0382**	**0.0316**	0.0147	**0.0212**	0.0067	0.0096	0.0095	-0.0033	0.0156	0.0094	0.0056					
13	-0.0020	-0.0013	0.0016	-0.0014	-0.0040	-0.0027	-0.0003	0.0093	0.0007	-0.0032	-0.0003	0.0262				
14	0.0032	0.0018	*0.0139*	0.0071	0.0055	0.0070	0.0070	*0.0219*	0.0087	0.0091	0.0106	**0.0392**	0.0008			
15	0.0031	0.0036	0.0077	0.0058	0.0055	0.0064	0.0051	**0.0177**	0.0063	-0.0001	0.0098	**0.0411**	-0.0011	0.0048		
16	0.0085	0.0015	-0.0024	-0.0008	-0.0042	-0.0023	-0.0036	0.0007	-0.0033	-0.0026	-0.0026	0.0122	0.0022	0.0089	0.0071	
17	**0.0211**	**0.0213**	0.0091	0.0141	0.0015	0.0010	0.0044	-0.0026	0.0086	-0.0041	0.0053	0.0073	0.0124	**0.0266**	**0.0188**	**0.0155**

Table 4. Analysis of molecular variance (AMOVA) of mitochondrial DNA cytochrome oxidase I (659 bp) sequence variability among samples of giant red sea cucumbers *Apostichopus californicus* from the Northeastern Pacific. Sample location numbers as in Figure 1 and Table 1.

Sample location																	Divergence			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	F_{ST}	*P*	F_{SC}	*P*
[*	*	*	*	*	*	*	*	*	*	*	*	*]					0.0023	0.0770	–	–
[*	*	*]	[*	*	*	*	*	*	*	*	*	*]					0.0046	0.0322	0.0006	0.3518
[*	*	*]	[*]	[*	*	*	*	*]	[*	*	*	*]					0.0020	0.1880	0.0008	0.3052
			[*]	[*	*	*	*	*]	[*	*	*	*]					-0.0063	0.5501	0.0008	0.3742
				[*	*	*	*	*]	[*	*	*	*]					-0.0012	0.7634	0.0008	0.4446
									[*	*	*	*]				[*]	0.0012	0.6060	0.0070	0.0429
				[*	*	*	*	*	*	*	*	*]					–	–	0.0001	0.4342
[*	*	*	*	*	*	*	*	*	*	*	*	*]	[*	*	*]		0.0026	0.1153	0.0026	0.0355
													[*	*	*]		–	–	0.0069	0.0556

Table 5. Summary of diversity by locus and observed and adjusted (F_{ST}^{ENA}) global values of F_{ST} and range of null allele frequencies among samples of giant sea cucumbers *Apostichopus californicus* from the Northeastern Pacific Ocean. Range of null-allele frequencies estimated with the EM maximum likelihood algorithm in FreeNA (Chapuis and Estoup 2007) and ML-NULLFREQ (Kalinowski and Taper 2006). A_N = number of alleles. A_R = allelic richness. H_O = observed heterozygosity. H_E = expected heterozygosity assuming Hardy-Weinberg proportions.

						Range of null allele frequencies	
Locus	A_N	H_O	H_E	F_{ST}	F_{ST}^{ENA}	FreeNa	ML-NULLFREQ
Pca_Psc1	38	0.666	0.948	0.0028	0.0002	0.102–0.269	0.102–0.261
Pca_Psc2	33	0.340	0.778	0.0004	0.0015	0.325–0.490	0.305–0.466
Pca_Psc4	24	0.426	0.848	0.0042	0.0028	0.220–0.438	0.204–0.438
Pca_Psc5	12	0.508	0.643	0.0098	0.0087	0.068–0.239	0.059–0.153
Total	107	0.485	0.770	0.0041	0.0037	–	–

Table 6. Summary statistics for four microsatellite loci in samples of giant sea cucumbers *Apostichopus californicus*. Sample location numbers as in Table 1. N = sample size. A_N = number of alleles, excluding null alleles. A_R = allelic richness with minimal samples size of 52 sea cucumbers. A_{PRI} = private allelic richness per sample. H_O = observed heterozygosity averaged over four loci. H_E = expected heterozygosity averaged over four loci. F_{IS} = inbreeding coefficient (Weir and Cockerham 1984). Rho_{IS} = inbreeding coefficient based on allelic size and frequency. Parameter estimates without correction for null alleles.

Sample	Date	N	A_N	A_R	A_{PRI}	H_O	H_E	F_{IS}	Rho_{IS}
1	2011	89	17.8	16.1	0.16	0.494	0.805	0.377	0.175
2	2011	85	16.5	14.8	0.08	0.521	0.807	0.341	0.384
3	2011	52	16.0	16.0	0.01	0.507	0.809	0.361	0.382
4	2010	93	17.0	15.3	0.00	0.460	0.774	0.400	0.267
5	2010	80	19.0	17.2	0.02	0.449	0.820	0.439	0.051
6	2010	93	17.8	16.2	0.00	0.494	0.796	0.376	0.313
7	2010	93	18.0	15.9	0.08	0.487	0.814	0.395	0.385
8	2010	93	16.8	15.1	0.09	0.507	0.800	0.353	0.222
9	2010	82	17.8	16.1	0.47	0.468	0.801	0.403	0.300
10	2011	53	15.0	14.9	0.29	0.485	0.814	0.392	0.229
11	2011	92	19.0	16.5	0.26	0.469	0.794	0.402	0.319
12	2010	89	18.0	16.3	0.21	0.513	0.802	0.357	0.160
13	2010	94	17.5	15.6	0.16	0.487	0.804	0.380	0.093
14	2002	94	17.8	16.2	0.00	0.485	0.800	0.391	0.456
15	2001	81	19.8	17.0	0.55	0.496	0.799	0.372	0.427
16	2002	70	18.3	16.7	0.40	0.471	0.802	0.400	0.452
17	2002	85	16.3	15.4	0.46	0.438	0.788	0.443	0.559
Mean	–	83.4	17.6	16.0	0.19	0.484	0.802	0.387	0.304
Total	–	1418	107	–	–	0.485	0.824	0.389	0.303

Table 7. F_{ST} between samples of giant sea cucumbers *Apostichopus californicus* from the northeastern Pacific Ocean. Sample numbers correspond to those in Table 1. (a) Values calculated from observed genotypic frequencies at four microsatellite loci (Weir and Cockham 1984, ARLEQUIN). Italicized values indicate significant at $P = 0.05$, and bold values indicate significance after Bonferroni correction of 136 comparisons. (b) Values based on genotypic frequencies adjusted for null alleles with the ENA correction of Chapius and Estoup (2007) in program FreeNA.

(a)

	Sample number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	-0.0001															
3	-0.0015	0.0002														
4	0.0061	0.0036	0.0081													
5	0.0002	0.0040	0.0059	0.0032												
6	0.0022	-0.0006	0.0013	-0.0040	-0.0071											
7	0.0017	0.0006	0.0009	0.0013	0.0019	0.0001										
8	-0.0011	0.0021	-0.0009	*0.0067*	0.0028	0.0002	-0.0002									
9	0.0034	0.0055	0.0007	*0.0199*	*0.0117*	*0.0139*	*0.0069*	0.0035								
10	0.0037	*0.0076*	0.0027	0.0202	*0.0111*	*0.0136*	0.0059	0.0062	-0.0024							
11	-0.0016	0.0011	-0.0009	0.0092	*0.0084*	0.0048	0.0001	-0.0008	0.0010	0.0011						
12	0.0130	0.0139	0.0063	0.0307	**0.0237**	*0.0284*	*0.0174*	*0.0119*	0.0002	0.0002	0.0058					
13	0.0083	0.0081	0.0072	*0.0183*	0.0083	*0.0133*	*0.0094*	0.0065	0.0028	0.0014	0.0047	0.0070				
14	*0.0101*	0.0093	0.0059	**0.0225**	*0.0166*	*0.0207*	*0.0098*	0.0071	-0.0001	-0.0019	0.0034	0.0010	0.0050			
15	0.0095	*0.0085*	0.0032	*0.0205*	*0.0181*	*0.0156*	*0.0092*	0.0058	0.0013	0.0031	0.0039	0.0005	0.0026	0.0011		
16	-0.0021	0.0016	-0.0005	*0.0081*	*0.0077*	0.0025	-0.0034	0.0009	0.0023	0.0027	0.0005	*0.0094*	0.0049	0.0021	0.0056	
17	*0.0179*	*0.0191*	*0.0114*	**0.0346**	*0.0268*	*0.0333*	*0.0232*	*0.0154*	0.0038	0.0050	*0.0087*	0.0020	0.0067	0.0065	0.0023	*0.0153*

Table 7. Continued

(b)

	Sample number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	0.0011															
3	0.0011	0.0010														
4	0.0042	0.0026	0.0044													
5	0.0042	0.0022	-0.0001	0.0016												
6	0.0109	0.0061	0.0104	0.0022	0.0019											
7	0.0111	0.0046	0.0078	0.0035	0.0009	-0.0005										
8	0.0059	0.0049	0.0054	0.0033	-0.0003	0.0008	0.0007									
9	0.0148	0.0104	0.0128	0.0084	0.0034	-0.0011	-0.0002	0.0020								
10	0.0106	0.0032	0.0054	0.0020	0.0022	0.0004	-0.0011	0.0032	0.0018							
11	0.0126	0.0070	0.0125	0.0052	0.0049	-0.0012	0.0001	0.0030	-0.0006	0.0001						
12	0.0158	0.0099	0.0149	0.0066	0.0073	-0.0004	0.0031	0.0052	0.0012	0.0009	-0.0007					
13	0.0051	0.0007	0.0049	0.0013	0.0009	0.0016	0.0014	0.0008	0.0032	0.0020	-0.0001	0.0035				
14	0.0048	0.0007	0.0031	-0.0001	-0.0013	0.0017	0.0014	0.0005	0.0048	0.0018	0.0041	0.0067	-0.0005			
15	0.0033	0.0022	0.0019	0.0018	0.0016	0.0011	0.0045	0.0000	0.0055	0.0031	0.0040	0.0042	0.0011	0.0014		
16	0.0046	0.0034	0.0030	0.0039	-0.0001	0.0010	0.0036	-0.0005	0.0016	0.0049	0.0023	0.0044	-0.0014	-0.0002	-0.0004	
17	0.0189	0.0123	0.0171	0.0092	0.0085	0.0026	0.0031	0.0076	0.0013	0.0008	0.0009	0.0010	0.0058	0.0071	0.0104	0.0078

Table 8. Analysis of molecular variance (AMOVA) of microsatellite allele-frequency variability among samples of giant red sea cucumbers *Apostichopus californicus* from the Northeastern Pacific. Sample location numbers as in Figure 1 and Table 1. Analysis was made on allelic frequencies unadjusted for null alleles, except that PCR failures were coded as null-allele homozygotes.

Sample location																	Divergence			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	F_{ST}	P	F_{SC}	P
[*	*	*	*	*	*	*	*	*	*	*	*	*]					0.0076	<0.0001	–	–
[*	*	*]	[*	*	*	*	*	*	*	*	*	*]					0.0064	0.0027	0.0053	0.0011
[*	*	*]	[*]	[*	*	*	*	*]	[*	*	*	*]					0.0047	<0.0001	0.0041	0.0600
			[*]	[*	*	*	*	*]	[*	*	*	*]					0.0020	0.0670	0.0035	0.1673
				[*	*	*	*	*]	[*	*	*	*]					0.0020	0.0672	0.0035	0.1721
									[*	*	*	*]				[*]	0.0026	0.1941	0.0037	0.2303
[*	*	*	*	*	*	*	*	*	*	*	*	*]	[*	*	*]		0.0000	0.7839	0.0070	<0.0001
													[*	*	*]		–	–	0.0033	0.2928

Figure 1

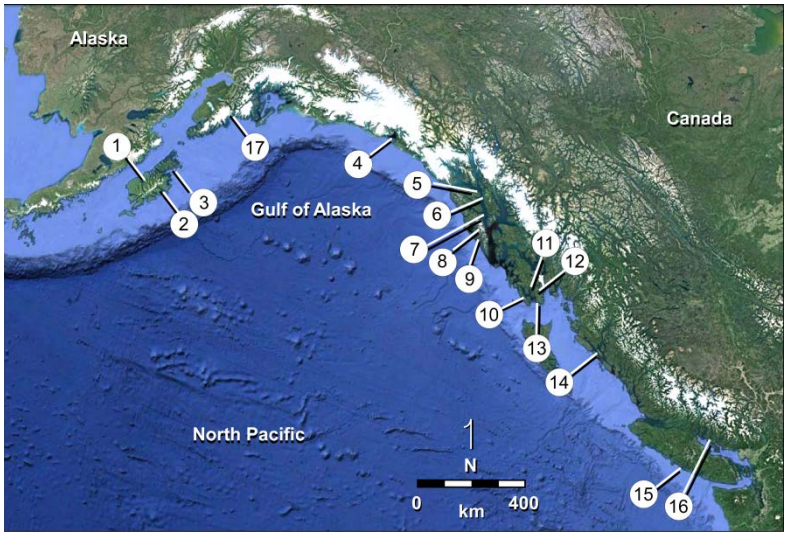

Alaska
Canada
Gulf of Alaska
North Pacific
N
0
km
400
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

Figure 2

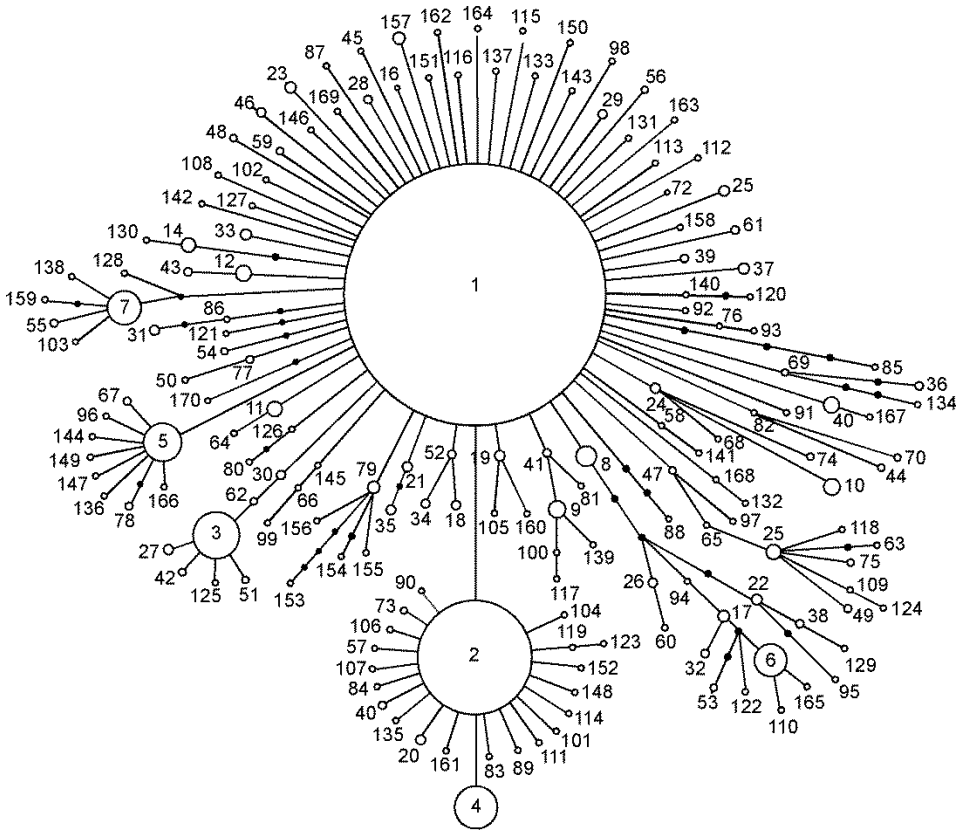

1
2
3
4
5
6
7
157 162 164 115 150 98 56 163 112 25 61 37 120 93 85 36 134 167 70 44 10 74 141 168 132 97 65 88 47 8 41 9 139 100 117 105 160 19 52 18 34 21 35 79 145 66 156 99 62 30 80 64 126 11 170 50 77 54 121 86 31 43 12 33 14 130 128 138 159 55 103 144 96 67 166 78 136 147 149 27 42 125 51 153 154 155 90 73 106 57 107 84 40 135 20 161 83 89 111 101 114 148 152 123 104 119 60 94 26 22 17 38 49 124 109 75 118 63 25 129 95 165 110 122 53 32 24 58 82 91 40 69 39 140 92 76 116 137 133 143 29 131 113 72 158 16 151 28 169 146 59 102 127 23 87 45 48 108 142

Figure 3

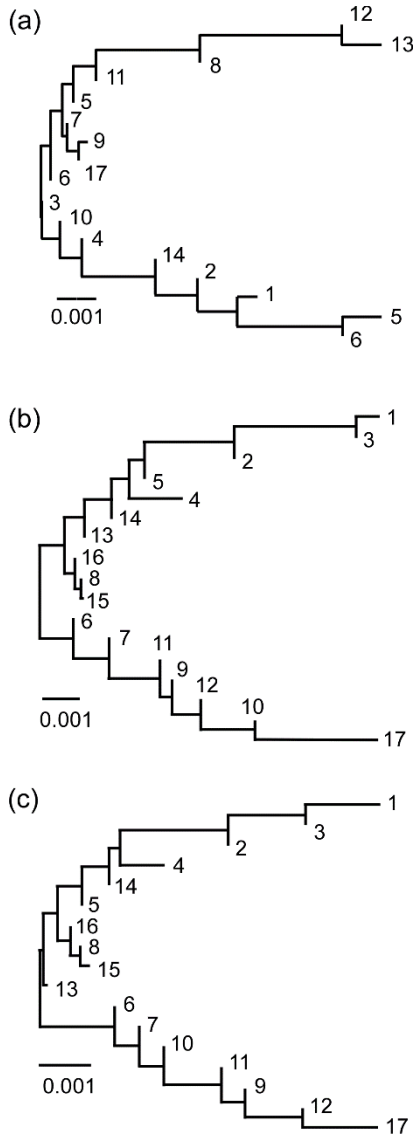
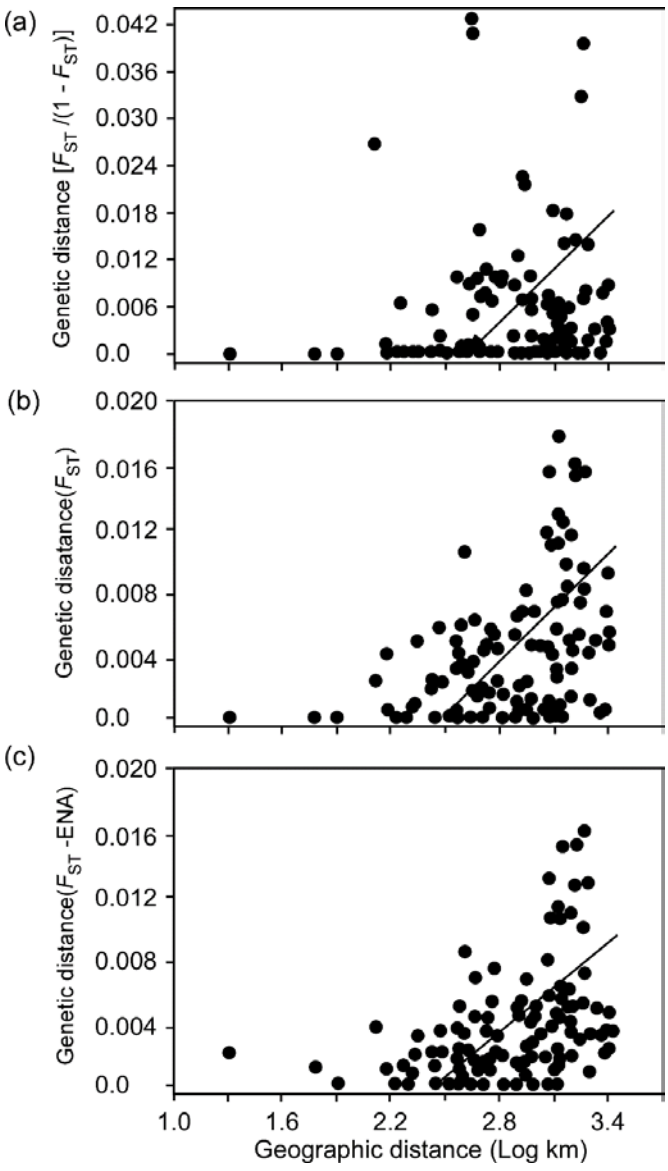

(a)
12
13
8
11
5
7
9
6
17
3
10
4
14
2
1
0.001
5
6
(b)
1
3
2
5
4
14
13
16
8
15
6
7
11
9
12
10
0.001
17
(c)
1
3
2
4
14
5
16
8
15
13
6
7
10
11
9
0.001
12
17

Figure 4


(a)
Genetic distance [F_{ST} /(1 - F_{ST})]
0.042
0.036
0.030
0.024
0.018
0.012
0.006
0.0
(b)
Genetic disatance(F_{ST})
0.020
0.016
0.012
0.008
0.004
0.0
(c)
Genetic distance(F_{ST} -ENA)
0.020
0.016
0.012
0.008
0.004
0.0
1.0
1.6
2.2
2.8
3.4
Geographic distance (Log km)