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FINAL REPORT

Stock Structure of Aleutian Island Golden King Crab

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ABSTRACT:

The delineation of population boundaries is a crucial component of the harvest management of wild populations. Here, we report the of a survey of genetic variability among populations of golden king crabs (*Lithodes aequispinus*) along the Aleutian Islands, in the Bering Sea and southeastern Alaska. These crabs are captured in deep waters with baited pots. An analysis of sequence variability along 643 base pair segment of mitochondrial DNA ND5 gene in 517 crabs revealed no overall departure from neutrality and a total haplotype diversity of $h = 0.670$ without any geographic trend. An AMOVA detected significant haplotype-frequency differences among populations ($F_{ST} = 0.344$, $P < 0.0001$), which were due to differences between populations on a scale of tens to hundreds of kilometers within regions ($F_{SC} = 0.314$, $P < 0.0001$). The analysis of 14 microsatellite loci in 1044 crabs revealed only moderate levels of heterozygosity ($H_O = 0.605$ to 0.692) and allelic richness ($A_R = 4.07$ to 8.00) in populations. Significant allele-frequency differentiation appeared overall 19 samples ($F_{ST} = 0.002$, $P = 0.003$). This heterogeneity was due to differentiation between Aleutian Island populations as a group and those in southeastern Alaska ($F_{ST} = 0.003$, $P = 0.0001$). However, no significant differentiation was detected between populations within the two regions. Additional analyses including isolation by distance and STRUCTURE did not detect heterogeneity among populations within either region. These genetic results support the present practice of managing regional Aleutian Island and southeastern Alaska populations separately, but do not support separate management units within the two regions. Nevertheless, harvest management should account for small-scale structure among golden king crab populations.

KEY WORDS:

Genetic population structure · Fishery management · Mitochondrial DNA · Microsatellite DNA

CITATION:

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INTRODUCTION:

The goal of this study was to use mitochondrial (mt) DNA and microsatellite DNA markers to search for genetic differences in Golden King Crab (GKC) across the Aleutian Island archipelago to improve definitions of stock structure in GKC. A particular fisheries management concern is whether GKC populations along the Aleutian Islands need to be split into multiple units for stock assessments. Our null hypothesis is that GKCs along the Aleutians represent a panmictic population.

OBJECTIVES:

1) Delineate population production units of golden king crab in Alaska waters with molecular genetic markers.

2) Estimate migration rates between populations of golden king crabs with the spatial distributions of genetic markers.

3) Estimate level of genetic diversity and genetic effective population sizes of golden king crab stocks.

All objectives were completed through sample collection and DNA extraction and subsequent analyses.

CHAPTER 1:

Phylogeography and Management of Golden King Crab populations in Alaska

Abstract:

The delineation of population boundaries is a crucial component of the harvest management of wild populations. Here, we report the of a survey of genetic variability among populations of golden king crabs (*Lithodes aequispinus*) along the Aleutian Islands, in the Bering Sea and southeastern Alaska. These crabs are captured in deep waters with baited pots. An analysis of sequence variability along 643 base pair segment of mitochondrial DNA ND5 gene in 517 crabs revealed no overall departure from neutrality and a total haplotype diversity of $h = 0.670$ without any geographic trend. An AMOVA detected significant haplotype-frequency differences among populations ($F_{ST} = 0.344$, $P < 0.0001$), which were due to differences between populations on a scale of tens to hundreds of kilometers within regions ($F_{SC} = 0.314$, $P < 0.0001$). The analysis of 14 microsatellite loci in 1044 crabs revealed only moderate levels of heterozygosity ($H_O = 0.605$ to 0.692) and allelic richness ($A_R = 4.07$ to 8.00) in populations. Significant allele-frequency

differentiation appeared overall 19 samples (F_{ST} = 0.002, P = 0.003). This heterogeneity was due to differentiation between Aleutian Island populations as a group and those in southeastern Alaska (F_{ST} = 0.003, P = 0.0001). However, no significant differentiation was detected between populations within the two regions. Additional analyses including isolation by distance and STRUCTURE did not detect heterogeneity among populations within either region. These genetic results support the present practice of managing regional Aleutian Island and southeastern Alaska populations separately, but do not support separate management units within the two regions. Nevertheless, harvest management should account for small-scale structure among golden king crab populations.

Introduction:

An understanding of population structure in needed to optimize the extent of individuals included in stock assessments (Ward 2006). When the geographic extent of a stock is large and includes small independent populations along with large populations, the smaller populations may be overharvested, leading to the loss of productivity and possibly to the loss of unique genetic diversity. Fishery stocks have largely been identified with morphological and physical tags to measure connectivity between populations and demographic analyses to resolve independent trajectories among populations (e.g. Zheng et al. 1996). Genetic markers can also contribute to defining stocks, but with the recognition that the processes influencing genetic variability generally operate on longer time scales than those producing ecological variability (Waples and Gaggiotti 2006; Waples et al. 2008). When genetic differences are detected between populations, ecological differences are also likely to be present. However, the lack of genetic differentiation between populations may occur even when ecological differences are present.

In this study, we used genetic markers to attempt to improve the understanding of stock structure among golden king crab (GKC; *Lithodes aequispinus*) in Alaska. These crabs inhabit deep coastal waters of the North Pacific from northern British Columbia, throughout the Gulf of Alaska and Bering Sea, along the Aleutian Archipelago, into Russian coastal waters to as far as central Japan (Butler and Hart 1962; Rodin 1970; Hiramoto 1985; Somerton and Otto 1985; Otto and Cummiskey 1985). Populations in the Bering Sea and along the Aleutian Archipelago are jointly managed by the Alaska Department of Fish and Game (ADF&G) and the North Pacific Fishery Management Council (NPFMC), and those in the Gulf of Alaska are managed by

ADF&G. Catches are limited within regions defined by harvest registration areas and are limited to males. Quotas are determined from size-age profiles that indicate abundance trends and from abundance survey data examined with population models (Zheng et al. 1996). The accuracies of these models, however, depend on correctly defining discrete, more or less self-sustaining populations.

The biology of GKC, together with oceanic features of the Aleutian Islands and southeastern Alaska, suggests that populations may be genetically subdivided. The first is that larval development in GKC is lecithotrophic (development without feeding) so that larvae are not tied phytoplankton production in upper waters (Shirley and Zhou 1997; Long and Van Sant 2015). Hence surface ocean currents may not be important in dispersing larvae and connecting populations through gene flow. Additionally, tagging data show that adults do not move long distances, only a few tens of kilometers at most (Blau and Pengilly 1994; Watson and Gish 2000). Effort and catch by the commercial fisheries indicate that GKC occurs in pockets of abundance, suggesting the possibility of isolated subpopulations. Lastly, the topography of the coastline may reinforce isolation between subpopulations. In southeastern Alaska, subpopulations are isolated in deep fjords. In this area, red king crab subpopulations show strong genetic differences between bays (Grant and Cheng 2012). Along the Aleutians, subpopulations may be isolated by deep underwater canyons located between islands (Penguilly 2012). All of these features may contribute to a subdivided population structure.

The use of genetic variation in and among populations to inform the management of harvested populations is well established. Genetic markers have been used to resolve population structure Alaskan populations of snow (Albrecht et al. 2014), Tanner crabs (Bunch et al. 1998), and red king crabs (Grant and Cheng 2012; Vulsteck et al. 2013). The results of studies of red king crab (RKC; *Paralithodes camtschaticus*) are particular useful for framing the expectations for GKC because of similarities in geographical distribution and life histories. Previous studies of RKC showed three large genetic subdivisions across the North Pacific (Grant et al. 2014). A western group included populations extending from Japan and Russia to the northern Bering Sea and the outer Aleutian Islands. A second group included populations in the southeastern Bering Sea and western Gulf of Alaska, and a third group included populations inhabiting the fjords of southeastern Alaska. Populations within the first two groups showed little genetic divergence from one another, but populations in the fjords of southeastern Alaska were generally genetically

distinctive from one another (Grant and Cheng 2012; Vulsteck et al. 2013). RKC populations in southeastern Alaska additionally showed much reduced levels of genetic diversity, likely due to frequent local extinctions and colonizations.

In contrast to RKC, GKC adults inhabit deep waters, so that patterns of adult GKC movement and planktonic larval dispersal differ from those of RKC, and hence patterns of connectivity between populations may also differ. Average size and size at maturity of GKC decrease at higher latitudes (Somerton and Otto 1985), and northern females are also more fecund than southern females for a given size. However, it is unclear whether these biological differences are due to local environmental influences or to genetic differences between populations in cold and temperate habitats. Mean size also decreases with depth in some areas, suggesting an ontogenetic shoreward migration (Somerton and Otto 1986). Adult GKC migrate into shallower waters to mate as RKC do, but appear to have a more protracted breeding season lasting from late winter to fall, or perhaps all year (Otto and Cummiskey 1985; Somerton and Otto 1986).

The goal of this study was to use mitochondrial (mt) DNA and microsatellite DNA markers to search for genetic differences between populations that might be used to improve definitions of stock structure in GKC. A particular management concern is whether GKC populations along the Aleutian Islands should be treated as a single unit for stock assessments. Our null hypothesis is that GKCs along the Aleutians represent a panmictic population. We test this hypothesis with measures of genetic divergence between subpopulations and ANOVA-like tests of mtDNA and microsatellite markers, and inferences based on assumptions of Hardy-Weinberg proportions and linkage equilibrium between microsatellite loci. We found support for a partition between populations along the Aleutian Islands and southeastern Alaska, but no support for regional east-west differences along the Aleutians. However, small-scale mtDNA differences appeared between subpopulations within regions.

Materials and Methods:

Sample collection and DNA extraction

Samples were collected in Southeast Alaska during stock assessment cruises conducted by Alaska Department of Fish and Game and by commercial fishermen during commercial harvests

in along the Aleutian Islands. Leg muscle and hemolymph was collected from crabs during research cruises and hemolymph on commercial boats. Samples were preserved in 95% ethanol until DNA extraction. DNA was extracted from tissues with NucleoSpin® 96 Tissue (MachereY-Nagel Inc., Bethlehem, PA).

Mitochondrial DNA PCR amplification and sequencing

We initially surveyed mtDNA cytochrome oxidase I (COI) for genetic variability. A segment of the cytochrome oxidase subunit I (COI) was amplified with PCR using the universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). PCR cocktails consisted of a 50 µL mixture of 2.0 µL templates DNA in 1x Colorless GoTaq Flexi Buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3 µM of forward and reverse primers, and 1U GoTaq Flexi DNA polymerase. PCR amplification were conducted in ABI 9700 thermocyclers with an initial denaturation of 1 min at 95^{o}C, 37 cycles of 40 sec at 95^{o}C, 40 sec at primer annealing temperature 41^{o}C, and 1 min at 72^{o}C; the final cycle was at 4^{o}C for 5min. The PCR amplifications were sequenced in the forward and reverse directions by Genewiz Inc. (South Plainfield, NJ). An initial survey showed low levels of diversity, so we used another mtDNA gene ND5 for the population study.

Partial sequences of mtDNA NADH dehydrogenase subunit 5 (ND5) were amplified with PCR and the forward and reverse primer pair (5'- AAACCCTGGCCTCCTAAGGT-3' and 5'-GCGCCGGGGTAGTAATTCAT),which were designed from golden king crab GenBank mtDNA sequences. The PCR was conducted in a 50 µL reaction mixture containing 2 µL of templates DNA in 1x Colorless GoTaq Flexi buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3 µM of forward and reverse primers, and 1U GoTaq Flexi DNA polymerase. Cycling conditions consisted of an initial denaturation of 1 min at 95^{o}C, followed by 5 cycle of 1 min at 94^{o}C, 1 min 30 sec at 45^{o}C annealing temperature, and 1min 30 sec at 72^{o}C. In subsequent cyclers, 37 cycles of 1 min denaturation at 94^{o}C, 1min 30 sec at 50^{o}C annealing temperature, and 1 min 30 sec extension at 72^{o}C, ending with a final extension for 5 min at 72^{o}C. The PCR amplifications were sequenced in the forward and reverse directions by Genewiz Inc. (South Plainfield, NJ). Representatives from different extraction plates were re-extracted and sequenced to check for handling errors. Forward and reverse sequences for all individuals were aligned and edited with

MEGA 7.0.20 (Kumar et al. 2016) and Finch TV 1.4.0 (Geospiza Inc.) to produce a 643 base pair sequences of ND5 for population analysis.

Microsatellite genotyping

We used a suite of 14 microsatellite loci that had been developed for red king crabs (*Paralithodes chamtschatica*), including *Pca101*, *Pca103*, *Pca107* (Seeb et al. 2002; Vulstek et al. 2013), *Pca100B* (Vulstek et al. 2013), and blue king crabs (*Paralithodes platypus*) *Ppl08*, *Ppl12*, *Ppl16*, *Ppl17*, *Ppl18*, *Ppl19*, *Ppl21*, *Ppl32*, *Ppl37*, and *Ppl40* (Stoutamore et al. 2012). The polymerase chain reaction (PCR) was used to amplify 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), 1.5 mM $MgCl_2$ (Promega Inc. Madison, WI), 0.20 mM of each nucleotide (Applied Biogsytems, Inc.), 0.4 µM of forward and reverse primers, 0.05 U GoTaq Flexi DNA polymerase (Promega Inc. Madison, WI) and deionized water. Hot start PCR was used to amplify *Ppl32*, *Ppl08*, and *Ppl40*. In these PCR reactions, AmpliTaq Gold® DNA Polymerase with Buffer I (Applied Biosystems, Inc.) replaced GoTaq Flexi Buffer and DNA polymerase. Optimal thermal cycling profiles varied among loci (Table S1). Microsatellites were fractionated by size in an Applied Biosystems 3730 capillary DNA sequencer. Genotypes were scored with GeneMapper 5.0 (Applied Biosystems). Eight percent of the samples were re-extracted and genotyped for quality control.

Statistical analyses

We used TCS 1.21 (Clement et al. 2000) to construct a 95% plausible parsimony network of mtDNA haplotypes. ARLEQUIN 3.5.2.2 (Excoffier and Lisher 2010) was used to estimate haplotype (h), and nucleotide (θ_π) diversities and to test for departure from neutrality with Tajima's (D_T) (Tajima 1989) and Fu's (F_S) (Fu 1997) statistics with 10,000 randomizations to assess significance. Several summary statistics, including the number of polymorphic nucleotide sites (N_{poly}), number of private haplotypes in a sample (N_{priv}), number of haplotypes (N_h), number of expected haplotypes given neutrality (N_{exp}), the numbers of transitions (*ts*) and tranversions (*tv*) were also calculated with ARLEQUIN. Pairwise estimates of divergence were estimated with F_{ST} (Weir and Cockerham 1984) which ranges between 0.0 (identity) and 1.0 (complete

dissimilarity), with 10,000 randomizations to estimate the probability that $F_{ST} > 0.0$. Isolation by distance (IBD) between populations was tested with a Mantel's test between F_{ST} and shore-line geographic distances between pairs of samples (Kimura and Weiss 1964). Geographic distances were estimated in kilometers with the path function in Google Earth. IBD was tested with Mantel's correlations between differences matrices with the online IDBWS facility (Jensen et al. 2005) We used the analysis of molecular variation (AMOVA) in ARLEQUIN to explore geographical structure with several geographic models of population structure.

Microsatellite genotypes among samples were examined with several statistics. First, we used MICRO-CHECKER (van Oosterhout et al. 2004) to check for stutter, large-allele dropout and null alleles. Since none of these conditions were indicated, we proceeded with the following analyses. GENEPOP 4.6 (Rousset 2008) was used to estimate several summary statistics, including observed (H_O) and expected (H_E) heterozygosities and the inbreeding coefficients F_{IS} (based on genotypic frequencies) and Rho_{IS}. (based on allelic frequencies and repeat distances between alleles). The number of alleles (A_N) and allelic richness (A_R) were calculated with HP-RARE (Kalinowski 2005) with a minimal sample size of 60 genes.

Population structure was assessed with pairwise F_{ST} (Weir and Cockerham 1984) with 10,000 randomizations to test the probability that $F_{ST} > 0.0$ in ARLEQUIN. A neighbor-joining tree (Saitou and Nei 1987) was constructed from pairwise values of F_{ST} with MEGA 7. Isolation by distance between populations was tested with a Mantel's test between F_{ST} and shore-line geographic distances between pairs of samples. Geographic distances were estimated in kilometers with the path function in Google Earth. Mantel's tests were made in IDBWS online (Jensen et al. 2005). Several models of population structure based on geography were tested with an AMOVA analysis in ARLEQUIN. The probability that F_{ST} (divergence between groups of populations) and F_{SC} (divergence between subpopulations within groups) was greater than 0.0 was estimated with 10,000 randomizations.

Lastly, we used STRUCTURE (Pritchard et al. 2000) to test for population structure on the basis of Hardy-Weinberg proportions and linkage disequilibrium between loci. We used a burn-in of 5,000 iterations and runs of 50,000 iterations for each of 10 replicates for $K = 1$ to 10 subpopulations. Runs were made without prior information about the locations of samples. We used the ΔK method of Evanno et al. (2005), which is implemented in STRUCTURE

HARVESTER (Earl and vonHoldt 2012) to estimate the number of genetically distinctive populations.

Results:

Mitochondrial DNA sequence variability

A 643 base-pair (bp) segment of the mtDNA ND5 gene was sequenced in 517 golden king crabs from 13 localities extending from the central Bering Sea, along the Aleutian Island and to the fjords of southeastern Alaska (Figure 1, Table 1). Most samples were collected from 2011 to 2016, but some samples dated to the mid-1990s. A total of 19 polymorphic nucleotide sites with a transition-transversion ratio of 5.33 defined 25 haplotypes (Table 2). A central haplotype occurred in 54% of the crabs examined (Figure 2) and numerous additional haplotypes occurred in frequencies of 0.02 to 13%. The expected number of haplotypes under neutrality (9.52) was much less than the observed number (25) and was due to numerous low-frequency haplotypes. This excess of low-frequency haplotypes produced a significant value of F_S = -8.583 (P = 0.024), but not of D_T = -0.586 (P = 0.318) (Table 3). Gene diversities ranged from h = 0.325 to 0.803 among samples and was h = 0.670 overall (Table 3). Nucleotide diversities ranged from θ_π = 0.00053 to 0.00415 and was θ_π = 0.00329 overall. Gene diversity was slightly larger, on average, in the Aleutian samples (h = 0.588) than in the samples from southeastern Alaska (h = 0.537). A similar trend appeared for the average value of nucleotide diversity between Aleutian Island samples (θ_π = 0.00258) and southeastern Alaskan samples (θ_π = 0.00172).

Geographic structure between populations was examined with several approaches. First, we tested for isolation by distance over all samples and among samples separately along the Aleutian Islands and in southeastern Alaska and found no significant correlation between genetic and geographic distance in these comparisons (Figure 3a). Second, we estimated genetic distances between populations. These genetic distances varied from F_{ST} = 0.0 between 10 pairs of samples to F_{ST} = 0.656 between samples 1 and 13. After Bonferroni correction for multiple comparisons, 40 of the 78 F_{ST} values were significant. A lack of geographic correspondence in the distribution of genetic distances between samples was apparent in a neighbor-joining tree of the F_{ST} values (Figure 4a). Third, the results of the AMOVA comparisons reflected these results. Significant haplotype frequency heterogeneity was detected over all samples (F_{ST} = 0.0342, P <0.0001), but a comparison between samples from the Aleutian Islands (N = 7) and southeastern

Alaska (N = 6) was not significant (F_{ST} = 0.081, P = 0.150) (Table 5). However, significant heterogeneity appeared among samples within the two regions (F_{SC} = 0.311, P <0.0001). Accordingly, no isolation by distance between populations was apparent overall (r = 0.116, P = 0.138), among the Aleutian Island samples (r = -0370, P = 0.952), or among samples from southeastern Alaska (r = -0.270, P = 0.621) (Fig. 3a). A comparison between three samples from the Adak Island area collected in 1994, 2002, and 2016, showed significant haplotype-frequency differences overall (F_{ST} = 0.448, P <0.0001), and between each of the three sample comparisons (P <0.0078) (Table 5).

Microsatellite variability

A total 14 microsatellite loci with repeat sequence motifs of 3 and 4 nucleotides were genotyped in 1088 to 1131 crabs, depending on locus (Table 6). The sequence repeat motive among loci ranged from 2 to 4 with two loci, Lae_Pca107 and Lae_Pca116, showing imperfect dinucleotide insertions into quadra-nucleotide repeats. MICRO-CHECKER indicated the lack of null alleles or large-allele drop out. The number of alleles ranged from 3 to 27 among loci. Locus heterozygosities ranged from 0.108 (Lae_Pca119) to 0.885 (Lae_Pca108). Three loci (Lae_Pca101, Lae_116, Lae_Pca119) showed significant allele-frequency heterogeneity among samples.

Genetic diversity and inbreeding were estimated in several ways. Sample sizes range from 5 to 100 among 19 samples and averaged 58.3 per locality (Table 7). No apparent geographic trends appeared in the distribution of genetic diversity among samples. The number of alleles per locus on average ranged from A_N = 4.31 to 8.43 and averaged 7.23 among samples. Allelic richness varied from A_R = 4.07 to 8.00 and averaged 6.99. The lowest value was in a sample consisting of only 5 crabs. Observed average heterozygosities over the 14 loci ranged from H_O = 0.605 to 0.692 and averaged 0.638. Expected heterozygosities were marginally larger, ranging from H_E = 0.645 to 0.681 and averaging 0.656. The inbreeding coefficient, F_{IS} (based on observed and expected genotypic frequencies) ranged from -0.008 to 0.104 and averaged 0.037 among samples. Total F_{IS} for pooled samples was 0.035. The coefficient Rho_{IS} (based on genotypic frequencies and the number of sequence repeats between alleles) ranged from -0.094 to 0.088 and average -0.007. Total Rho_{IS} for pooled samples was 0.013.

Genetic population structure was estimated from sample allelic frequencies in three ways. First, we tested for isolation by distance overall and within regions, along the Aleutian Islands and within southeastern Alaska. The overall comparison including the 19 samples was significant (r = 0.469, P <0.0001), but not in tests for isolation by distance within each region (Figure 3). Second, pairwise estimates of genetic divergence ranged from F_{ST} = 0.0 (including negative values) to 0.086 (Table 8). Eight of the 171 comparisons between samples were significant, but none of the F_{ST}'s were significant after a Bonferroni correction for multiple comparisons. A neighbor-joining tree of F_{ST} values did not show any geographic clusters of samples (Figure 4b). Third, we used AMOVAs detect possible partitions among pooled groups of samples that may not have been apparent in pairwise values of F_{ST} (Table 9). An overall test among the 19 samples detected significant differences with F_{ST} = 0.002 (P = 0.003). The source of this heterogeneity was allele-frequency differences between samples from the Aleutian Islands and Bering Sea, and samples from southeastern Alaska (F_{ST} = 0.003, P <0.0001). No significant differences were detected among samples from the Aleutians Islands, or among samples from southeastern Alaska. Temporal comparisons between samples from the Adak Island regions were not significant. Fourth, the STRUCTURE analysis testing for 1 to 10 subpopulations indicated that only a single genetic population existed that included both the Aleutian Islands and southeastern Alaska (Figures 5, 6). The results are illustrated for a-priori assumptions of two (Figure 7a) and three (Figure 7b) populations. Vertical lines represent individual crabs and vertical black bars delineate sample locations.

Discussion:

Our survey of genetic variability within and among populations of golden king crabs revealed several novel features of genetic population structure. We examined 517 crabs in 13 samples for mtDNA variability and 1044 crabs in 19 samples for microsatellite variability. Microsatellites in invertebrates are commonly plagued by null alleles that result from mutations in the PCR primer binding regions. However, no null alleles were detected in the GKC microsatellite dataset. The geographic coverage of samples and samples sizes should be adequate to detect genetic population structure in golden king crabs if it exists (Hale et al. 2012).

Both sets of markers show that the levels and distributions of genetic variability among populations differ considerably from those in red king crabs. Red king crab populations show

strong differences in levels of genetic diversity with populations in southeastern Alaska having reduced diversity (mtDNA: $h \approx 0.250$; microsatellites $H \approx 0.770$) compared to western populations ($h \approx 0.600$; $H \approx 0.850$) (Grant and Cheng 1012; Vulstek et al. 2013). In contrast, genetic diversity is consistently low across the range of GKC in the Northeastern Pacific (mtDNA: $h = 0.557$; microsatellites: $H = 0.638$) (Tables 3, 7).

The results of the survey of genetic variability show only weak, but significant, differentiation between regional groups. Isolation by distance appeared in the overall distribution of microsatellite allelic frequencies, but not in the distribution of mtDNA haplotype frequencies (Figure 3). Microsatellite markers showed significant differentiation between populations in the Aleutian Island and in southeastern Alaska ($F_{ST} = 0.003$, P <0,0001) (Table 9), but mtDNA markers did not ($F_{ST} = 0.078$, $P = 0.157$) (Table 5). However, mtDNA showed significant differences between populations within both regional groups (Aleutian Island: $F_{ST} = 0.349$, P <0.0001; southeastern Alaska: F_{ST} =0.227, P <0.0001). A comparison between western and eastern populations across the Aleutian Island was not significant ($F_{ST} = 0.0$, P =0.502) for mtDNA, as was a comparison of northern and southern populations in southeastern Alaska ($F_{ST} = 0.0$, $P = 0.868$). Tests of heterogeneity at the 14 microsatellite loci did not provide support for genetic population structure among Aleutian Island populations ($F_{ST} = 0.0004$, $P = 0.514$) (Table 9). No population structure appeared in the analysis with the program STRUCTURE (Figures 5–7). Hence, no groups of populations along the Aleutian Island or in southeastern Alaska could be identified that merited status as distinct management unit.

The contrast between mtDNA and microsatellite DNA variability in resolving the genetic population structure of golden king crabs may be due to a combination of several mechanisms. Microsatellites are expected to provide considerable resolution of population structure because microsatellite loci are characterized by high mutation rates that should track recent population events (Karl et al. 2012). Four mechanisms, working solely or in combination, may be responsible for this discordance between markers in estimates of genetic population structure: 1) sex-biased natural selection, 2) microsatellite allelic homoplasy, 3) contrasting effective population sizes between organellar and nuclear genes, and 4) sex-biased dispersal. One possibility is that selection pressures differ between sexes so that one sex survives better in non-natal habitats. This mechanism appears to explain sex-specific differences in mtDNA variability among populations of brown mussels (*Perna perna*) (Teske et al. 2012). In this case, contrasting

values of N_e between marker types could be eliminated as a potential source of the differences in population structure between males and females. Male mussles appeared to tolerate environmental conditions in non-natal habitats better than females, who appear to be adapted to local conditions. In golden king crabs, males and females may experience the same environment differently because females carry clutches of developing eggs for several months (Long and Van Sant 2015). However, little is known about possible physiological differences between males and females and their responses to environmental variables.

Second, convergence in microsatellite allelic states may reduce the apparent allele-frequency differences between populations. Microsatellite mutations arise during reproduction largely by stepwise increases or decreases in short nucleotide-sequence repeats that define the size of an allele. A particular allele, however, can arise from either an increase in the number of repeats in a smaller allele, or a decrease in the number of repeats in a larger allele. When mutation rates are large, this converge on the same sized allele tends to homogenize allele frequencies and obscure any underlying population structure. While we cannot completely discount this mechanism, microsatellites have nevertheless been instrumental in detecting fine-scale population structure in numerous marine species (e.g. red king crab, Vulstek et al. 2013).

A third explanation invokes differences in the effective population sizes (N_e) between mitochondrial and nuclear genes. The matrilineal inheritance of organellar mtDNA produces an effective population size (N_e) that is about one quarter of that for nuclear-encoded microsatellites (Ballard and Whitlock 2004). Greater amounts of random drift in female lineages should produce greater haplotype-frequency divergences between local populations than would be expected for bi-parentally inherited microsatellite loci. Smaller N_e leads to greater levels of random drift of matrilineal haplotypic frequencies in small local populations experiencing frequent bottlenecks in size or periodically experiencing extinctions and colonizations as part of a larger metapopulation. In addition to geographical shifts in allele frequencies, metapopulation effects are also expected to produce uneven genetic diversities among populations (Hanski and Gilpin 1997). The distribution of diversity among samples supports this latter hypothesis. The number of mtDNA haplotypes varied from 2 to 13 among samples, and haplotype diversities ranging from $h = 0.232$ to 0.822 (Table 3). Variation in sample size does not explain these differences. This mechanism was used to explain mito-nuclear discordance in population structure in a marine goby (*Pomatoschistus minutus*) (Larmuseau et al. 2010).

A fourth possibility is that sex-biased dispersal leads to different apparent population structures between marker types (Greenwood 1980). Since the mode of inheritance differs between matrilineal mtDNA variants and bi-parentally inherited microsatellites, differences in the degree of dispersal between sexes can lead to discordance in estimates of population structure. For example, gravid females GKC crabs may be less prone to dispersing than are males. Sex-biased dispersal has been invoked to explain differences between marker types in such diverse organisms as horseshoe crabs (King et al. 2005), sea turtles (Fitzsimmons et al. 1997), and whales (Lyrholm et al. 1999). For GKC, there are no tagging data or genetic samples with individuals identified by sex (Goudet et al. 2002) that could be used to test this possibility.

In conclusion, the results of this survey of genetic variability in populations of golden king crabs showed little support for regional differences between western and eastern population groups along the Aleutian Islands or among north-south population groups in southeastern Alaska. However, significant heterogeneity was detected between populations on geographic scales of tens of kilometers with mtDNA markers, but not with microsatellite markers. This points to classical metapopulation structure, in which small subpopulations are sufficiently isolated to produce genetic differences between the subpopulations. The overall implications for harvest management are that populations along the Aleutian Island chain and in southeastern Alaska each consist of a single genetic metapopulation. There is no genetic support for subdividing these populations into groups for separate stock assessments. However, if golden king crabs are in fact distributed in small subpopulations that may be susceptible to local extinctions and recolonizations, harvests should be diffusive and should not be focused on small areas that might jeopardize these small subpopulations (DiCosimo et al. 2010). The management of the genetic dimension of wild populations is an important factor in the sustainable exploitation of wild populations (Kenchington et al. 2003).

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Table 1. Sample locations, sample size, north latitude and longitude, depth and date

Location		Latitude	Longitude	Date
Aleutian Islands				
1	Amchitka-Semisopochnoi islands	51.245 to 52.081	179.174 to 179.951	February 2016
2	Amatignak-Ulak islands	51.391 to 52.038	-179.968 to -178.912	February 2016
3	Gareloi-Tanaga islands	51.449 to 51.724	-178.871 to -178.355	February 2016
4	Adak Island, landed catch	51.863	-176.661	1994
5	Adak Island, landed catch	51.770	-178.330	2002
6	Adak Island area	51.560 to 51.836	-177.804 to -176.908	February 2016
7	Atka Island area	51.821	-174.426	February 2016
8	Amlia-Seguam Islands	52.144 to 52.580	-171.091 to -172.921	August 2015
9	Yunaska–Samalga Islands	52.423 to 52.542	-169.617 to -170.579	August 2015
10	Dutch Harbor, landed catch	53.901	-166.513	1995
Bering Sea				
11	Bowers Ridge	53.981 to 54.440	179.640 to 179.992	February 2016
12	Central Bering Sea	57.537	-178.242	February 2016
13	South of Pribilof Islands	58.200	-158.600	1996
Southeastern Alaska				
14	Lower Lynn Canal	58.242	-134.944	2011

15	Stephens Passage	58.079	-134.088	2011
16	Frederick Sound	57.050	-134.250	1996
17	Mid Chatham Strait	56.826	-134.533	2011
18	Lower Chatham Strait	56.554	-134.573	2011
19	Clarence Strait	56.167	-132.764	2011

Table 2. Haplotype frequencies of mitochondrial DNA ND5 (643 bp) in samples of golden king crab (*Lithodes aequispina*) (*n* = 517) from the Aleutian Islands, Bering Sea, and Northeastern Pacific. Sample numbers correspond to those in Table 1 and Figure 1.

	Sample												
Haplotype	1	4	6	8	10	12	13	14	15	16	17	18	19
1	41	7	10	41	18	5	14	26	30	19	23	23	22
2	4		4	5	7		5	7	8	4	6	11	6
3		16	16	1	8		7		1	15			
4	2	1	4		6	1	16			6		3	
5		13	2							1			
6	1	1	7		1		3			2			
7		3	3										
8									2				2
9			1		1		4						
10	1	1				2							
11			1				1						
12									1				
13								1					
14			1										
15		1											
16		1											
17		1											
18		1											
19		1											
20		1											
21										1			
22										1			
23					1								
24					1								
25	1												

Table 3. Summary statistics of mitochondrial DNA ND5 sequence (643 bp) analysis of golden king crabs (*Lithodes aequispina*) (*n* = 517) from the Aleutian Islands and southeastern Alaska. Location numbers correspond to those in Table 1 and Figure 1.

Sample	*N*	N_{poly}	N_{priv}	N_h	N_{Exp}	*ts*	*tv*	*h*	±SE	θ_π%	±SE	D_T	*P*	F_S	*P*
Aleutian Islands															
1	50	5	1	6	2.44	4	1	0.325	±0.084	0.078	±0.075	-1.341	0.073	-3.275	0.011
4	48	12	5	13	9.44	10	2	0.803	±0.037	0.369	±0.227	-0.364	0.400	-3.815	0.050
6	50	9	1	10	10.40	7	2	0.822	±0.035	0.410	±0.247	0.878	0.833	-0.818	0.397
8	47	6	0	3	1.92	5	1	0.232	±0.076	0.063	±0.066	-1.806	0.010	-0.076	0.375
10	43	7	1	8	7.69	6	1	0.760	±0.045	0.370	±0.228	1.283	0.909	-0.054	0.535
Bering Sea															
12	8	3	0	3	2.90	2	1	0.607	±0.164	0.144	±0.126	-0.813	0.272	0.071	0.426
13	50	7	0	7	9.24	5	2	0.795	±0.031	0.345	±0.216	1.140	0.882	0.719	0.676
Southeastern Alaska															
14	34	5	0	3	2.63	5	0	0.383	±0.086	0.089	±0.082	-1.407	0.065	0.418	0.542
15	42	8	0	5	3.29	6	2	0.461	±0.082	0.142	±0.112	-1.436	0.055	-0.524	0.369
16	49	8	2	8	7.62	7	1	0.747	±0.039	0.415	±0.249	0.329	0.917	0.574	0.656
17	29	1	0	2	2.32	1	0	0.340	±0.090	0.053	±0.060	0.528	0.818	0.919	0.520
18	37	3	0	3	3.81	2	1	0.533	±0.064	0.114	±0.097	0.053	0.954	1.034	0.706
19	30	2	0	3	2.88	2	0	0.432	±0.094	0.072	±0.072	-0.183	0.396	-0.101	0.358
Mean	39.8	5.8	0.8	5.7	5.12	4.7	1.1	0.557	±0.713	0.189	±0.143	-0.241	0.506	-0.379	0.532
Total	517	19	-	25	9.52	16	3	0.670	±0.020	0.329	±0.203	-0.586	0.318	-8.583	0.024

N = sample size; N_{poly} = number of polymorphic nucleotide sites; N_{priv} = number of private substitution; N_h = number of haplotypes; N_{exp} = expected number of haplotypes; *ts* = number of transitions; *tv* = number of transversions; *h* = gene diversity (standard error); θ_π = nucleotide diversity (standard error) presented as percentage; D_T = Tajima's measure of neutrality and probability: F_S = Fu's measures of neutrality and probability.

Table 4. Pairwise values of F_{ST} (lower triangle) and probability (upper triangle) for partial sequences of mitochondrial ND5 in samples of golden king crabs (*Lithodes aequispinus*) from the Aleutian Islands, Bering Sea, and southeastern Alaska.

1	–	<0.001	<0.001	0.694	<0.001	<0.001	0.221	0.121	0.288	<0.001	0.107	0.022	0.200
2	0.6562	–	0.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
3	0.4915	0.0751	–	<0.001	<0.001	0.007	0.001	<0.001	<0.001	0.061	<0.001	<0.001	<0.001
4	-0.0090	0.6674	0.5078	–	<0.001	<0.001	0.070	0.459	0.359	<0.001	0.313	0.028	0.319
5	0.1956	0.3374	0.1371	0.2163	–	0.099	0.159	<0.001	<0.001	0.157	<0.001	<0.001	<0.001
6	0.3481	0.2773	0.0789	0.3744	0.0254	–	0.022	<0.001	<0.001	0.166	<0.001	<0.001	<0.001
7	0.0337	0.4882	0.2944	0.0972	0.0475	0.1777	–	0.062	0.235	0.052	0.188	0.054	0.027
8	0.0178	0.6336	0.4701	-0.0031	0.1833	0.3423	0.0975	–	0.785	<0.001	1.000	0.378	0.931
9	0.0043	0.6061	0.4347	0.0019	0.1396	0.2923	0.0213	-0.0155	–	<0.001	0.495	0.419	0.786
10	0.3197	0.2094	0.0384	0.3371	0.0180	0.0147	0.1396	0.3040	0.2643	–	<0.001	<0.001	<0.001
11	0.0293	0.6488	0.4887	0.0045	0.2078	0.3636	0.1794	-0.0251	-0.0030	0.3270	–	0.367	0.754
12	0.0567	0.6253	0.4567	0.0543	0.1612	0.3126	0.1117	0.0025	-0.0026	0.2934	0.00709	–	0.391
13	0.0145	0.6362	0.4714	0.0023	0.1848	0.3379	0.1239	-0.0242	-0.0159	0.3066	-0.02323	-0.0037	–
	1	2	3	4	5	6	7	8	9	10	11	12	13

Table 5. Analyses of molecular variance (AMOVA) of temporal and spatial variability in mitochondrial DNA ND5 sequences (643 bp) among 12 samples of golden king crabs (*Lithodes aequispina*) along the Aleutian Islands and southeastern Alaska. Sample groupings for various AMOVAs are indicated by asterisks and brackets. Sample numbers as in Table 1 and Figure 1. F_{ST} is differentiation between population groups enclosed in brackets. F_{SC} is differentiation among populations within groups. *P* is the probability that F_{ST} or F_{SC} are significantly greater than 0.0.

Comparison													AMOVA			
1	4	6	8	10	13	14	15	16	17	18	19		F_{ST}	*P*	F_{SC}	*P*
[*	*	*	*	*	*	*	*	*	*	*	*]		0.344	<0.0001	–	–
[*	*	*	*	*	*]	[*	*	*	*	*	*]		0.078	0.157	0.314	<0.0001
[*	*	*	*	*	*]								0.349	<0.0001	–	–
[*	*	*]	[*	*	*]								-0.019	0.502	0.357	<0.0001
						[*	*	*	*	*	*]		0.227	<0.0001	–	–
						[*	*]	[*	*	*	*]		-0.058	0.868	0.250	<0.0001
	[*	*]											–	–	0.071	<0.0001

Table 6. Summary statistics for microsatellite variability among 19 samples of golden king crab from the North Pacific and Bering Sea. *N* is sample size for each locus. A_N is the number of alleles. A_P is the number of alleles occurring in only one sample (sometimes in multiple copies). *h* is heterozygosity per locus of pooled samples. *P* is the probability of exact G-test of allele-frequency differences between samples for each microsatellite locus. Default values of 10,000 burn-in, 20 batches of 5000 iterations each. No null alleles, or large-allele dropouts were detected with MICRO-CHECKER.

Locus	*N*	Repeat motif	A_N	A_{Pri}	*h*	Heterogeneity *P*	*S.E.*
Lae_Pca100B	1088	3	11	2	0.662	0.294	0.046
Lae_Pca101	1109	4	6	0	0.640	0.004	0.003
Lae_Pca103	1102	3	27	2	0.851	0.713	0.053
Lae_Pca107	1106	4 with dinuc mutations	19	3	0.781	0.278	0.046
Lae_Pca108	1100	4	24	4	0.885	0.125	0.034
Lae_Pca112	1131	4	3	0	0.597	0.338	0.025
Lae_Pca116	1125	4 with dinuc mutations	19	5	0.660	0.034	0.024
Lae_Pca117	1107	3	15	1	0.829	0.359	0.050
Lae_Pca118	1110	4	12	1	0.818	0.294	0.048
Lae_Pca119	1125	2	3	0	0.108	0.037	0.008
Lae_Pca121	1111	4	8	2	0.606	0.221	0.044
Lae_Pca132	1098	4	5	0	0.552	0.238	0.037
Lae_Pca137	1119	4	3	0	0.413	0.078	0.021
Lae_Pca140	1123	4	10	2	0.779	0.310	0.055

Table 7. Summary results of variability at 14 microsatellite microsatellite loci in 19 samples of golden king crabs from the Bering Sea and Northwestern Pacific. *N* is sample size. A_N is the average number alleles across loci. A_R is allelic richness. H_O is observed and H_E expected heterozygosity averaged over 14 loci. F_{IS} is the inbreeding coefficient based on allelic frequencies. Rho_{IS} is the inbreeding coefficient based on allelic size in addition to allelic frequencies.

Sample	*N*	A_N	A_R	H_O	H_E	F_{IS}	Rho_{IS}
1	67	7.64	7.46	0.641	0.668	0.042	0.037
2	59	7.64	7.62	0.659	0.655	-0.005	-0.065
3	60	7.07	7.05	0.664	0.659	-0.008	0.006
4	87	7.93	7.63	0.627	0.652	0.027	0.043
5	58	7.43	7.43	0.626	0.645	0.041	0.054
6	99	8.36	7.47	0.609	0.666	0.056	0.030
7	20	6.00	6.00	0.639	0.660	0.041	0.088
8	64	8.14	8.00	0.644	0.654	0.025	-0.094
9	76	8.14	7.75	0.629	0.660	0.039	0.058
10	100	8.43	7.67	0.631	0.652	0.044	0.017
11	59	7.07	7.07	0.636	0.681	0.025	-0.018
12	5	4.31	4.07	0.692	0.663	0.063	-0.079
13	100	8.36	7.54	0.647	0.658	0.025	0.022
14	38	6.43	6.43	0.657	0.648	0.002	-0.010
15	42	6.79	6.79	0.643	0.650	0.008	-0.094
16	109	8.36	7.48	0.628	0.661	0.035	0.019
17	29	6.50	6.50	0.605	0.647	0.085	-0.045
18	36	6.79	6.79	0.614	0.647	0.051	0.042
19	30	6.54	6.14	0.625	0.656	0.104	0.071
Mean	58.3	7.23	6.99	0.638	0.656	0.037	-0.007
Total	1044	11.71	10.72	0.633	0.656	0.035	0.013

Table 8. Pairwise F_{ST} values (lower triangle) and probabilities (upper triangle) between samples of golden king crabs (*Lithodes aequispinus*) from the Aleutian Islands (samples 1–13) and southeastern Alaska (samples 14–19). F_{ST} values were based on allelic frequencies at 14 microsatellite loci.

1	–	0.7692	0.0137	0.1743	0.0696	1.0	0.2747	0.4481	0.6846	0.9476	0.0904	0.8045	0.0207	0.0386
2	-0.0084	–	0.7907	0.3426	0.4186	1.0	0.2950	0.7833	0.8613	0.9975	0.9248	0.7643	0.4885	0.3112
3	0.0364	-0.0119	–	0.8815	0.2138	1.0	0.3368	0.6572	0.0862	0.9955	0.1607	0.6365	0.0403	0.0236
4	0.0136	0.0061	-0.0175	–	0.6515	1.0	0.4822	0.8978	0.4816	0.9953	0.6125	0.6856	0.1687	0.0066
5	0.0231	0.0028	0.0132	-0.0085	–	1.0	0.7661	0.5547	0.3496	0.9703	0.4483	0.6276	0.8646	0.0051
6	-0.0972	-0.1273	-0.1306	-0.1317	-0.1512	–	0.9962	1.0	1.0	1.0	1.0	0.9114	1.0	0.9894
7	0.0202	0.0203	0.0163	-0.0030	-0.0262	-0.1152	–	0.3900	0.4356	0.5792	0.3884	0.5752	0.2941	0.1331
8	0.0018	-0.0123	-0.0062	-0.0197	-0.0038	-0.1168	0.0086	–	0.1677	0.9642	0.1423	0.9176	0.2257	0.0407
9	-0.0046	-0.0143	0.0246	0.0001	0.0054	-0.1336	0.0071	0.0158	–	0.9999	0.7309	0.6789	0.3830	0.2040
10	-0.0151	-0.0359	-0.0296	-0.0326	-0.0244	-0.1237	-0.0100	-0.0224	-0.0408	–	0.8161	0.8358	0.6686	0.3520
11	0.0211	-0.0227	0.0180	-0.0069	0.0002	-0.0870	0.0064	0.0179	-0.0092	-0.0135	–	0.7944	0.1945	0.0619
12	-0.0832	-0.0714	-0.0296	-0.0675	-0.0446	-0.1888	-0.0218	-0.1403	-0.0424	-0.1223	-0.1016	–	0.7409	0.3498
13	0.0261	-0.0001	0.0276	0.0131	-0.0150	-0.1066	0.0156	0.0095	0.0037	-0.0055	0.0114	-0.0781	–	0.2677
14	0.0383	0.0108	0.0520	0.0645	0.0660	-0.0559	0.0432	0.0420	0.0180	0.0047	0.0367	0.0297	0.0102	–
15	0.0143	-0.0092	0.0715	0.0476	0.0110	-0.0934	0.0188	0.0255	0.0150	-0.0024	0.0091	-0.0205	0.0007	-0.0099
16	0.0284	-0.0051	0.0633	0.0383	0.0448	-0.0995	0.0108	0.0271	0.0162	-0.0074	0.0023	-0.0161	0.0145	-0.0095
17	0.0816	0.0476	0.0834	0.0611	0.0251	-0.0871	0.0135	0.0302	0.0646	0.0084	0.0280	-0.0424	0.0284	0.0617
18	0.0349	0.0067	0.0736	0.0310	0.0248	-0.0811	0.0138	0.0358	0.0227	0.0048	-0.0016	-0.0189	0.0146	-0.0014
19	0.0529	0.0336	0.0860	0.0420	0.0248	-0.0784	0.0013	0.0363	0.0141	-0.0119	0.0268	-0.0777	-0.0203	0.0161
	1	2	3	4	5	6	7	8	9	10	11	12	13	14

Table 8. Continued to the right.

1	0.2199	0.0120	0.0045	0.0703	0.0318
2	0.6706	0.6662	0.0797	0.4086	0.1655
3	0.0034	0.0002	0.0106	0.0082	0.0071
4	0.1041	0.0275	0.0083	0.0210	0.0806
5	0.2606	0.0033	0.1863	0.1421	0.1855
6	0.9995	1.0	0.9960	0.9987	0.9962
7	0.2839	0.3743	0.4324	0.3812	0.5412
8	0.1043	0.0325	0.1553	0.0790	0.1164
9	0.2316	0.1168	0.0232	0.1818	0.3377
10	0.4954	0.7715	0.3560	0.3764	0.6865
11	0.3069	0.4194	0.1753	0.5147	0.1845
12	0.5378	0.5702	0.7200	0.5933	0.8269
13	0.4539	0.0972	0.1400	0.2329	0.8378
14	0.5924	0.6912	0.0472	0.4808	0.3168
15	–	0.8507	0.378	0.5613	0.3244
16	-0.0158	–	0.2255	0.8658	0.3319
17	0.0095	0.0203	–	0.1320	0.5237
18	-0.0053	-0.0178	0.0474	–	0.676
19	0.0162	0.0121	0.0072	-0.0092	–
	15	16	17	18	19

Table 5. Analyses of molecular variance (AMOVA) of temporal and spatial variability in 14 microsatellite loci among 19 samples of golden king crabs (*Lithodes aequispina*) along the Aleutian Islands and southeastern Alaska. Sample groupings for various AMOVAs are indicated by asterisks and brackets. Sample numbers as in Table 1 and Figure 1. F_{ST} is differentiation between population groups enclosed in brackets. F_{SC} is differentiation among populations within groups. *P* is the probability that F_{ST} or F_{SC} are significantly greater than 0.0.

Comparison																			AMOVA			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	F_{ST}	*P*	F_{SC}	*P*
[	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*]	0.002	0003	–	–
[	*	*	*	*	*	*	*	*	*	*	*	*][	*	*	*	*	*	*]	0.003	<0.0001	0.001	0.330
[	*	*	*	*	*	*	*	*	*	*	*	*]							0.000	1.000	–	–
[	*	*	*	*	*	*][	*	*	*	*	*	*]							-0.0001	0.627	0.0006	0.258
													[*	*	*	*	*	*]	0.0004	0.514	–	–
													[*	*]	[*	*	*	*]	-0.0002	0.419	0.0005	0.480
			[*	*	*]														–	–	-0.001	0.843
			[*	*]															–	–	-0.000	0.534
			[*		*]														–	–	-0.002	0.928
				[*	*]														–	–	-0.001	0.757

Table S1. Polymerase chain reaction profiles for microsatellite loci in golden king carb (*Lithodes aequispinus*).

Locus	Thermal profile
Pca100B	92 °C/5 min; 32 cycles of (92 °C/30 sec + 56 °C/30 sec + 72 °C/20 sec); 72 °C 15 min
Pca101	95 °C/15 min; 32 cycles of (94 °C/30 sec + 54 °C/1 min 30 sec + 72 °C/1 min); 60 °C 15 min
Pca103	95 °C/15 min; 32 cycles of (94 °C/30 sec + 54 °C/1 min 30 sec + 72 °C/1 min); 60 °C 15 min
Pca107	92 °C/5 min; 32 cycles of (92 °C/30 sec + 56 °C/30 sec + 72 °C/20 sec); 72 °C 15 min
Ppl08	95 °C/5 min; 20 cycles of (95 °C/30 sec + 65 °C/30 sec + 72 °C/30 sec); 20 cycles of (95 °C/30 sec + 55 °C/30 sec + 72 °C/30 sec);72 °C 7 min
Ppl12	95 °C/5 min; 20 cycles of (95 °C/30 sec + 58 °C/30 sec + 72 °C/30 sec); 18 cycles of (95 °C/30 sec + 48 °C/30 sec + 72 °C/30 sec);72 °C 7 min
Ppl16	95 °C/5 min; 20 cycles of (95 °C/30 sec + 65 °C/30 sec + 72 °C/30 sec); 20 cycles of (95 °C/30 sec + 55 °C/30 sec + 72 °C/30 sec);72 °C 7 min
Ppl17	95 °C/5 min; 20 cycles of (95 °C/30 sec + 65 °C/30 sec + 72 °C/30 sec); 18 cycles of (95 °C/30 sec + 55 °C/30 sec + 72 °C/30 sec);72 °C 7 min
Ppl18	95 °C/5 min; 20 cycles of (95 °C/30 sec + 65 °C/30 sec + 72 °C/30 sec); 20 cycles of (95 °C/30 sec + 55 °C/30 sec + 72 °C/30 sec);72 °C 7 min
Ppl19	95 °C/5 min; 20 cycles of (95 °C/30 sec + 65 °C/30 sec + 72 °C/30 sec); 16 cycles of (95 °C/30 sec + 55 °C/30 sec + 72 °C/30 sec);72 °C 7 min
Ppl21	95 °C/5 min; 20 cycles of (95 °C/30 sec + 65 °C/30 sec + 72 °C/30 sec); 16 cycles of (95 °C/30 sec + 55 °C/30 sec + 72 °C/30 sec);72 °C 7 min
Ppl32	95 °C/5 min; 20 cycles of (95 °C/30 sec + 65 °C/30 sec + 72 °C/30 sec); 18 cycles of (95 °C/30 sec + 55 °C/30 sec + 72 °C/30 sec);72 °C 7 min
Ppl37	95 °C/5 min; 20 cycles of (95 °C/30 sec + 58 °C/30 sec + 72 °C/30 sec); 18 cycles of (95 °C/30 sec + 48 °C/30 sec + 72 °C/30 sec);72 °C 7 min
Ppl40	95 °C/5 min; 20 cycles of (95 °C/30 sec + 65 °C/30 sec + 72 °C/30 sec); 20 cycles of (95 °C/30 sec + 55 °C/30 sec + 72 °C/30 sec);72 °C 7 min

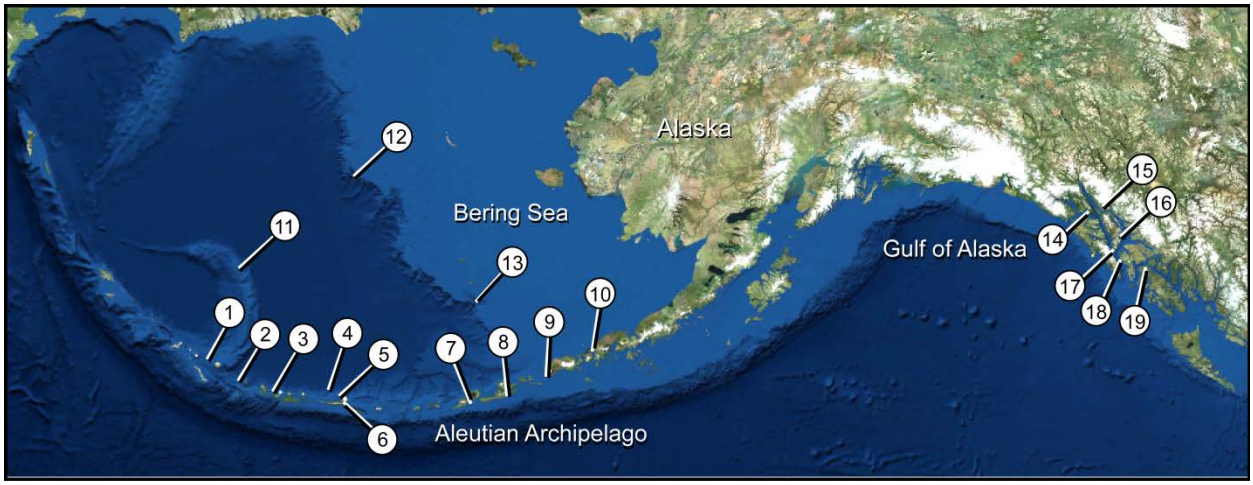

Figure 1. Map of Northeastern Pacific showing locations of samples used in study of genetic variability in golden king crab (*Lithodes aequispina*). Pointers indicate approximate locations of samples, which were pooled by area for statistical analysis. Sample numbers correspond to those in Table 1.

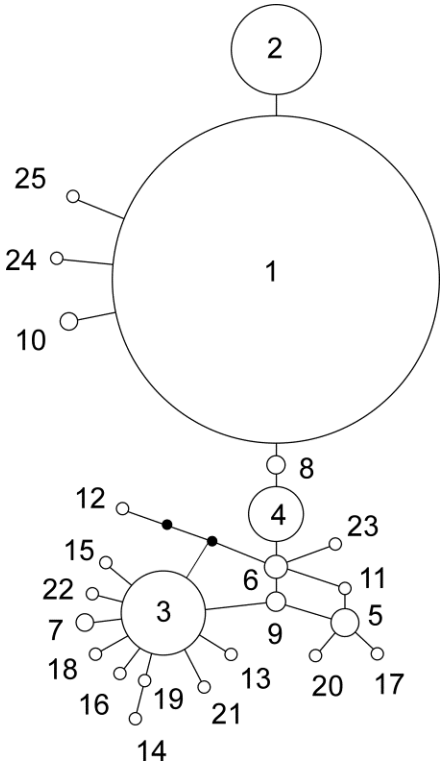

Figure 2. Haplotype network of mitochondrial DNA ND5 (bp = 643) of 517 golden king crabs (*Lithodes aequispina*) from the Bering Sea, Aleutian Islands and northeastern Pacific Ocean. Haplotype numbers correspond to those in Table 2.

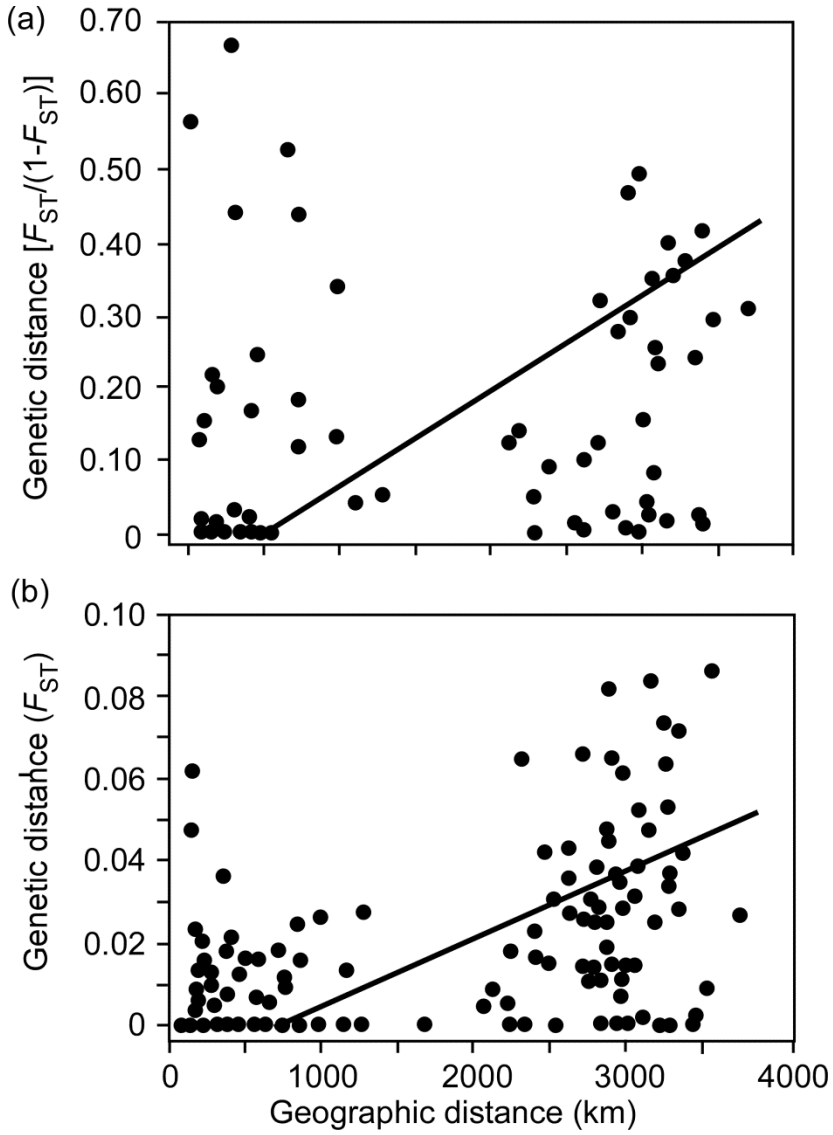

Figure 3. Isolation by distance (IBD) between populations of golden king crabs (*Lithodes aequispinus*) in the North Pacific Ocean. (a) IBD measured with mitochondrial DNA ND5; r = 0.116, P = 0.138. Aleutian populations only: n = 6, r = -0.370, P = 0.952. Southeastern Alaska populations only: n = 6, r = -0.270, P = 0.621. (b) IBD measured with 14 microsatellite loci; r = 0.469 , P < 0.0001. Aleutian populations only: n = 13, r = -0.054, P = 0.624. Southeastern Alaska populations only: n = 6, r = -0.206, P = 0.707.

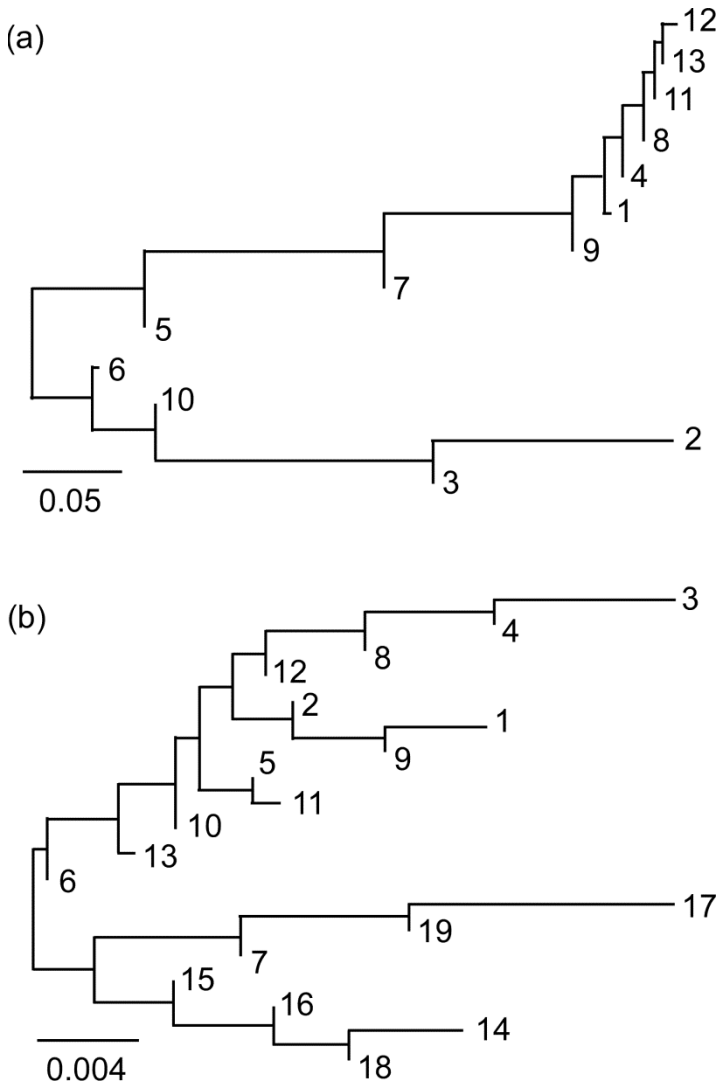

Figure 4. Neighbor-joining trees of genetic distances (F_{ST}) between samples of golden king crabs (*Lithodes aequispinus*) from the Aleutian Islands and southeastern Alaska. (a) mitochondrial DNA ND5 (643 bp). (b) 14 microsatellite loci. Negative values were zeroed to construct the trees. Sample numbers correspond to numbers in Figure 1 and Table 1.

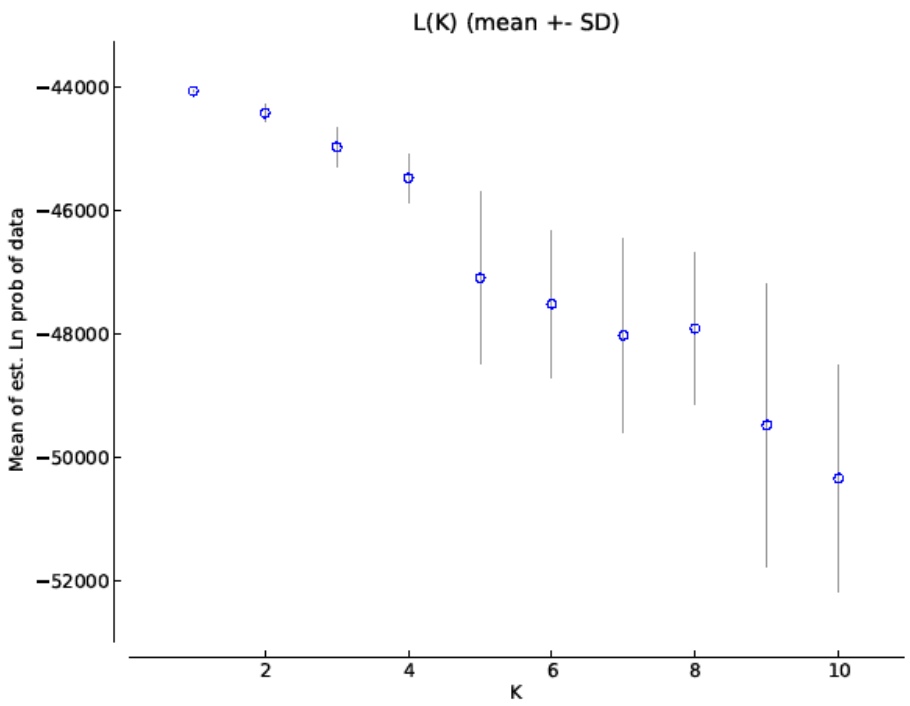

Figure 5. Means of and standard deviations of Log likelihoods of the number of genetically discrete subpopulations of golden king crab (*Lithodes aequispina*) in the Northeastern Pacific. STRUCTURE (Pritchard et al. 2000) was used to characterize population structure with 20 replicate runs each for K = 1 through 10 subpopulations, but without *a priori* assumptions about the number of subpopulations.

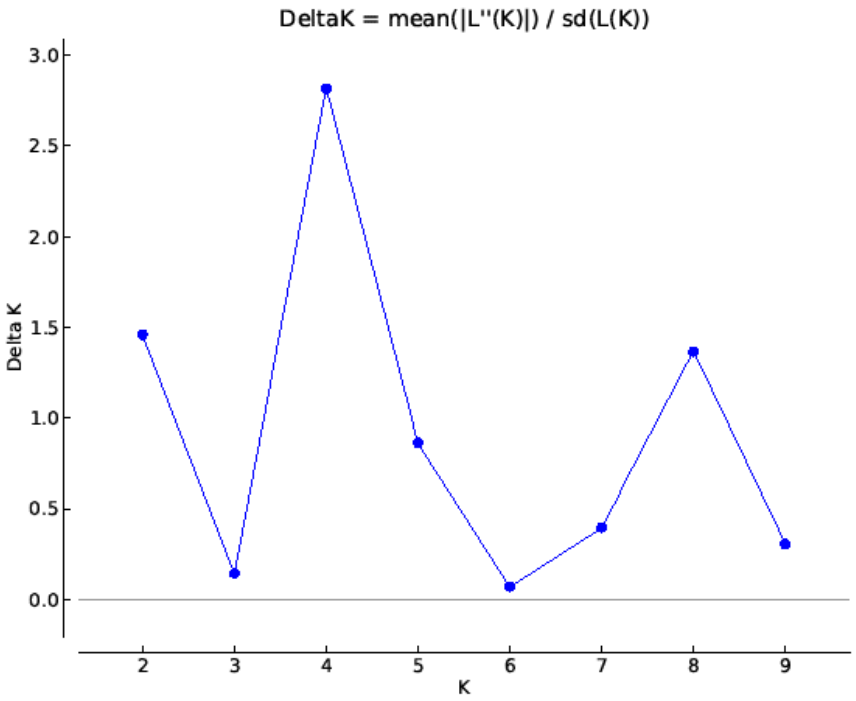

Figure 6. The delta-K method (Pritichard et al. 2000; Evanno et al. 2005) of estimating the number of genetically discrete subpopulations of golden king crab (*Lithodes aequispina*) in the Northeastern Pacific.

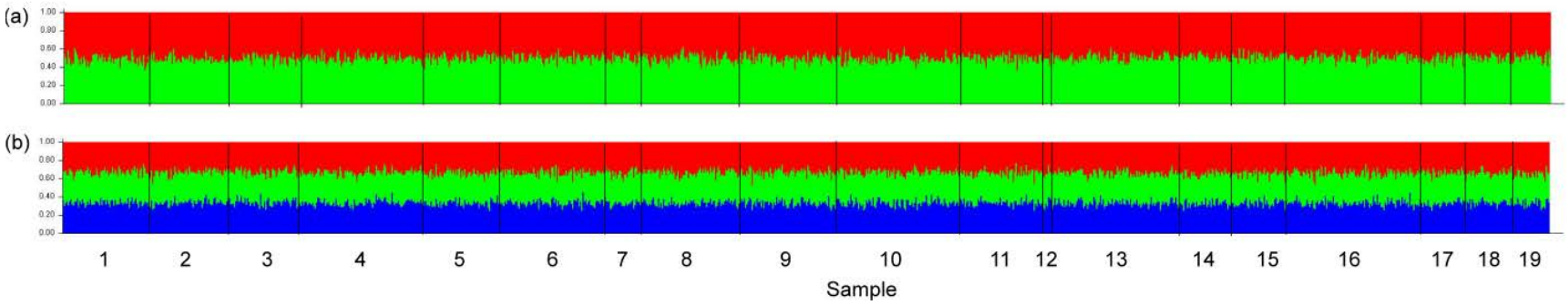

Figure 7. Analysis of 14 microsatellite loci to estimate without *a prior* information the number of genetically distinctive subpopulations of golden king crabs (*Lithodes aequispina*) in the Aleutian Islands, Bering Sea and Southeast Alaska. Histograms of the Q-matrix of likely membership in (a) two or (b) three subpopulations of 1144 individuals. Membership was estimated with STRUCTURE (Pritchard et al. 2000). Sample numbers correspond to those in Table 1 and Figure 1.

CONCLUSIONS:

The results of this survey of genetic variability in populations of golden king crabs showed little support for regional differences between western and eastern population groups along the Aleutian Islands or among north-south population groups in southeastern Alaska. However, significant heterogeneity was detected between populations on geographic scales of tens of kilometers with mtDNA markers, but not with microsatellite markers. This points to classical metapopulation structure, in which small subpopulations are sufficiently isolated to produce genetic differences between the subpopulations.

MANAGEMENT OR POLICY IMPLICATIONS:

The overall implications for harvest management are that populations along the Aleutian Island chain and in southeastern Alaska each consist of a single genetic metapopulation. There is no genetic support for subdividing these populations into groups for separate stock assessments.

DATA AND METADATA:

Data for this project will include all sample collection information (e.g., latitude/longitude, date/time) and all genotype data (mitochondrial and microsatellite) from approximately 1100 animals.

OUTREACH:

The results of this project were presented to the North Pacific Fishery Management Council's Crab Plan Team in January, 2018 and to the Science and Statistical Committee in February, 2018. Both groups were pleased with project results and the timeliness of them as the Aleutian Island Golden King Crab fishery regulations are under review/revision as this time and these results will be utilized to maintain the current split in the fishery into eastern and western (based on fishing effort not genetics). Additionally, this project and Alaska crab fisheries in general were discussed in person with the Adak school. C. Siddon met with teachers and students in February, 2016 at the school in Adak, AK. Unfortunately, due to vessel timing at the dock I was unable to get students down to handle/measure crab in person. However, through this outreach Siddon will continue to work with the school system during subsequent (annual) trips as Adak has become a more consistent location for offloading GKC for three of the commercial fishing vessels from August to March.

ACKNOWLEDGEMENTS:

We thank Wei Chang, Heather Hoyt, Heather Lillier and Zac Grauvogel for their assistance in generating the genotype data for this project. This project would not have been possible without the help of the commercial fishing industry. Specifically, we thank the vessel skippers (Rip Carlton, Chad Hoefer, Mark Medjo, Rick Alvarez, and Cory Cole) and their crew for providing access to animals across the entire Aleutian Island chain.