SPATIO-TEMPORAL POPULATION GENETIC STRUCTURE AND MATING SYSTEM OF RED KING CRAB (*Paralithodes camtschaticus*) IN ALASKA

By

Scott C. Vulstek

RECOMMENDED:

Dr. Ginny Eckert

Dr. Anthony Gharrett

Dr. David Tallmon, Advisory Committee Chair

Dr. Milo Adkison
Chair, Graduate Program in Fisheries Division

APPROVED:

Dr. Michael Castellini,
Dean, School of Fisheries and Ocean Sciences

Dr. Lawrence Duffy, Interim Dean of the Graduate School

Dec 6, 2011

Date

SPATIO-TEMPORAL POPULATION GENETIC STRUCTURE AND MATING SYSTEM OF RED KING CRAB (*Paralithodes camtschaticus*) IN ALASKA

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

By

Scott C. Vulstek, B.S.

Fairbanks, Alaska

December 2011

Abstract

Population genetic data can be used to make inferences about the population structure and mating system and inform management decisions of overharvested species such as red king crab (*Paralithodes camtschaticus*) in Alaska. Red king crab tissue and hemolymph samples were collected from 11 geographically distinct locations from Norton Sound through Southeast Alaska ($n = 845$). At six locations, two collections were taken at least one generation apart. Heterogeneity of allele frequencies over time was detected in three of the six locations examined, which suggested signs of recent population bottlenecks. An overall $F_{ST} = 0.025$ ($P < 0.001$) suggested moderate genetic differentiation among red king crab collections. A two-region model of spatial differentiation in which collections from Southeast Alaska diverge from those in the Gulf of Alaska and Western Alaska was supported by pairwise F_{ST} values, homogeneity tests, Bayesian clustering, and discriminant analysis of principal components. However, spatial analysis of molecular variance suggests some finer-scale structuring within regions and greater differentiation occurs among collections within Southeast Alaska than among the rest of the collections. In addition to population genetic structure analyses, 24 female red king crab and their broods were collected from the Bering Sea. Ovigerous females and 20 offspring per brood were genotyped in order to determine whether multiple mating of a female occurred. There was no evidence of multiple paternity in any brood. The results of this study support continued management of distinct geographic groups within the Gulf of Alaska/Western Alaska region and suggest that finer-scale management may be beneficial in Southeast Alaska.

Table of Contents

Page

Page

List of Figures

Page

List of Tables

Page

Acknowledgements

I would like to thank J.R. Guyon, P. Jensen, J.F. Morado, B. Daly and all those involved in the collection of samples for this project. Second, I would like to thank W.D. Templin, W.S. Grant, and T. Harrington of the Alaska Department of Fish and Game Gene Conservation Laboratory for providing historic samples. I am also very appreciative of my committee members, G.L. Eckert, A.J. Gharrett , and D.A. Tallmon, who have offered valuable guidance and suffered through countless drafts over the past few years. Finally, I would be remiss if I did not thank my fellow Tallmon Lab members, especially R. Kovach, T.P. Linderoth, and P.D. Barry. Their intellectual, technical, and moral support has been invaluable throughout this project.

Introduction

History of the Fishery

Red king crab (*Paralithodes camtschaticus*) play an important role in the economy and cultural identity of the people of Alaska. Red king crab have been a commercially important species in Alaskan waters since the 1930s when the Japanese fleet began harvesting crab in the eastern Bering Sea and near Kodiak Island in the Gulf of Alaska (Orensanz et al., 1998; Bowers et al., 2008; Bechtol and Kruse, 2009). United States fishing vessels began harvesting red king crab in the eastern Bering Sea in 1947, and catch per unit effort reached a maximum in 1960, although harvest continued to rise and eventually reached an annual peak of 129.9 million pounds in 1980 (Otto and Jamieson, 2001; Bowers et al., 2008; Dew, 2011). The economic value of the red king crab fishery was important to the development of coastal communities, such as Kodiak, and the current cultural and social importance of this species is evident in tourism, festivals, and mainstream media.

The collapse of red king crab populations in Alaska and their subsequent failure to rebound to historic abundances over the past few decades, despite cessation or reduction of commercial fishing (Wooster, 1992; Orensanz et al., 1998), highlight the need for a better understanding of the basic population structure, ecological interactions, and life history of this economically and culturally important species. Various factors have been proposed as causes for the failure of Alaskan red king crab populations, including overharvest as directed fishing and bycatch, climate change, predation, and disease (Orensanz et al., 1998; Dew and McConnaughey, 2005; Bechtol and Kruse,

2009). Dew and McConnaughey (2005) suggest that the collapse of the Bristol Bay fishery in the 1980s was primarily a result of directed male-only harvest followed by years of high female mortality as bycatch in yellowfin sole (*Limanda aspera*) and other groundfish trawl fisheries. Bechtol and Kruse (2009) reconstructed red king crab abundance, recruitment, and mortality around Kodiak Island from 1960 to 2004 and discovered that increased fishing pressure in the 1960s coincided with high levels of recruitment, which in turn led to unsustainable exploitation rates and diminishing recruitment pulses until the ultimate collapse of the fishery. Abundances of red king crab in Bristol Bay and around Kodiak Island are substantially lower than historic levels and, in the case of Kodiak Island, remain low even though the fishery has been closed since 1983 (Dew and McConnaughey, 2005; Bechtol and Kruse, 2009).

Population Genetic Structure

Difficulties associated with the assessment of spatial and temporal patterns of genetic differentiation among populations must be overcome in order to provide insight into the basic life-history of red king crab, such as larval dispersal and the location and approximate geographic scale of distinct mating subunits. High degrees of connectivity produce subpopulations that are genetically similar to each other, making population structure difficult to detect (Palumbi, 2004). Genetic divergence among large populations progresses slowly and can be reduced by the migration of only a few individuals per generation. Historical influences, such as post-glacial colonization, can also obfuscate divergence patterns. Marine fish and shellfish species with moderate to

extended pelagic larval stages (on the order of months) and relatively large population sizes, such as red king crab, often exhibit weaker genetic population structure than less abundant species and those with more sedentary larvae (Ward et al., 1994; Waples, 1998; Kinlan et al., 2005). This weak genetic structure previously led researchers to believe that many marine populations were essentially open, meaning that individuals were equally likely to mate with each other regardless of the geographic distances between them (Hauser and Carvalho, 2008). Advances in genetic techniques and increased knowledge of life history and dispersal patterns have led to the realization that many marine species do not have open populations as was once suspected. Rather, some marine species show high levels of population differentiation (Barber et al., 2002; Palumbi, 2004). Consequently, relationships between management boundaries and distinct genetic stocks may need to be reanalyzed (Palumbi, 1994; Strathmann et al., 2002; Sherman et al., 2008; Palof et al., 2011). The likelihood of detecting weak population structure in marine species may be increased by sampling larger numbers of loci or individuals, by using highly polymorphic markers, by acquiring temporally and spatially separated samples, and by coupling genetic data with information on life history, oceanography, and species ecology (Waples, 1998).

Previous studies of red king crab population structure in Alaska reported low to moderate levels of overall genetic divergence among subpopulations (F_{ST}) and suggested grouping populations into two or more regional subunits. Allozyme analyses by Seeb et al. (1989) and Grant et al. (2011) suggested grouping red king crab into three distinct regions: Southeast Alaska, Gulf of Alaska, and the Bering Sea. Grant et al. (2011)

observed an overall F_{ST} = 0.003 (±0.016) and no significant difference among temporally spaced samples within Bristol Bay and three locations within Southeast Alaska (Barlow Cove, Seymour Canal, and Deadman Reach) as determined by homogeneity tests (p > 0.05). Seeb et al. (2001) reported genetic differences between crabs sampled in Southeast Alaska and those from other collections in the Gulf of Alaska and the Bering Sea and hinted at some finer-scale structuring within the remaining samples, but results were based on observed allele frequency differences among samples at five microsatellite loci that likely included the presence of null alleles. Cheng et al. (in press) reported an overall F_{ST} = 0.045 from 15 single nucleotide polymorphisms (SNPs) for four populations of red king crab in Alaska.

Mating System

The genetic mating system of red king crab has not been explored and it is unknown whether female crabs mate with only a single male or are polyandrous during breeding. The mating process in red king crab and the lack of spermathecae in females (Powell and Nickerson, 1965) suggest that female crabs likely remain monandrous during a given mating season, but the true mating system of a species cannot always be predicted by physiology and mating behavior (Birkhead and Hunter, 1990; Chapman et al., 2004). Instances and rates of multiple paternity may shift as a result of highly skewed sex ratios or varying levels of predation (Gosselin et al., 2005; Neff et al., 2008).

Recently, researchers and regional stakeholders have begun to examine the feasibility of stocking red king crab and blue king crab (*P. platypus*) in Alaskan waters as

a supplement to natural populations and a potential method for overcoming recruitment limitation. Understanding the mating system as it relates to instances and rates of multiple paternity is then important for addressing issues of acceptable broodstock census size (N_c) and effective size (N_e). Mating system influences N_e, which is the size of an idealized population that would lose genetic variation at the same rate as the population in question. A large N_e decreases the rate of inbreeding and genetic drift and slows the accumulation of deleterious alleles . Females that are mated singly often produce less genetically diverse offspring than those mated multiple times in a given season. Mating system also influences the overall genetic variation present within broodstock family groups. The amount of genetic variation present in a group of individuals influences the number of genetic markers needed to discriminate that group within a larger population. This may be important when one wishes to monitor the survival, migration, and reproductive success of introduced individuals (Palsboll, 1999).

Objectives

The first objective of this research is to examine red king crab spatial and temporal population genetic structure with the use of polymorphic microsatellite loci. Comprehending patterns of genetic subdivision of a species in space and time is crucial for understanding historic population-level events as well as predicting future responses in the face of anthropogenic impacts and changing environmental conditions. The second objective is to determine whether female red king crab mate singly or multiply. This information regarding population genetic structure and mating system is particularly

important for red king crab in Alaska, because this information may contribute to improved management and subsequent chances of recovery for this commercially and culturally important species.

Materials and Methods

Sample Collection

A total of 845 red king crab individuals were collected from eleven locations spanning from Southeast Alaska to Norton Sound, and temporally spaced collections of individuals were obtained within Norton Sound, Bristol Bay, the Pribilof Islands, Barlow Cove, Gambier Bay, and Deadman Reach (Figure 1). Three broad geographic regions were defined *a priori* for population structure analyses: Western Alaska (including Adak Island, Norton Sound, Bristol Bay, and Pribilof Islands collections), Gulf of Alaska/Kodiak Island (Including Kachemak Bay, Chiniak Bay, and Alitak Bay collections), and Southeast Alaska (including Deadman Reach, Gambier Bay, Barlow Cove, and Seymour Canal collections). In 2008, individuals were sampled during Alaska Department of Fish and Game (ADFG) and NOAA National Marine Fisheries Service (NMFS) surveys. A maximum of 0.2 mL of hemolymph was collected from each individual crab with a 1.0 mL syringe, and hemolymph samples were placed in 95% ethanol in 96-well plates. Muscle tissue samples for all individuals collected from 1988-1996 were obtained from the ADFG Gene Conservation Laboratory in Anchorage, Alaska. These samples were originally collected from commercial fishing vessels or during stock surveys.

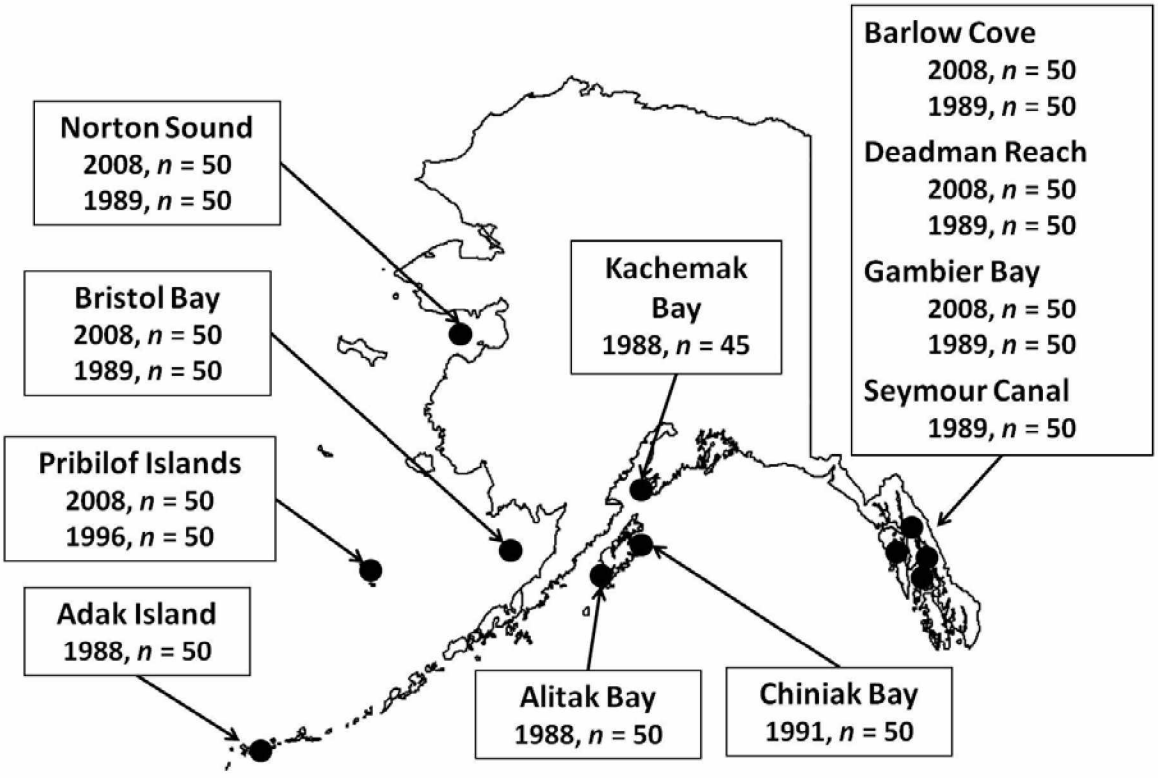

Figure 1. Red king crab sample locations and years. Sample sizes, locations, and years for spatial and temporal population genetic structure analyses.

Ovigerous female red king crab for mating system analysis were collected in 2007 and 2008 with pots by a commercial fishing vessel from Bristol Bay and shipped to the Alutiiq Pride Shellfish Hatchery in Seward, Alaska. Tissue samples were collected from the chelae of twelve female crabs for each year and a haphazard sample of 20 total embryos and zoeal stage 1 larvae (Z1) were taken from the brood of each female for genotyping and paternity analysis. Embryos and Z1 were grouped by female and sample date and preserved in 95% ethanol. Three previously developed red king crab microsatellite loci (*Pca*101, *Pca*103 and *Pca*107; Seeb et al., 2002) were used to determine mating system.

Microsatellite Analysis

Genomic DNA was extracted from hemolymph samples following the silica-based procedure of Ivanova et al. (2006) and stored at -20°C. A proteinase K and ammonium acetate precipitation technique (Puregene DNA™ isolation protocol - Gentra Systems, Minneapolis, MN) was used to extract genomic DNA from tissue samples and resulting samples were stored at -20°C. Six microsatellite loci were amplified for population genetic structure analyses using three previously designed primer pairs (*Pca*101, *Pca*103, and *Pca*104; Seeb et al., 2002), along with two redesigned primer pairs (*Pca*100B and *Pca*107B) and one newly designed primer pair (*Pca*201) (Table 1). Polymerase chain reaction (PCR) conditions and thermal profiles varied by microsatellite locus (Table 1).

Table 1. Red king crab primer characteristics and thermal profiles.

Locus	Primers	Sequence	Thermal Profile	GenBank no.
Pca100B	F: GGTGCTCATCATTACTCAGG R: TGTTGTTTCAGTTCGGGCT	$(TAA)_{11}$	92°C(5 min); 32 cycles of [92°C(30s)+56°C(30 sec)+1°C/s to 72°C+72°C(20s)]; 72°C(30min)	Not yet submitted
Pca101	F: TTTCGGTTACTCGATATAATGC R: TTTTTCTCTGCTTACGAAGG	$(TATC)_{18}AA(TCAA)_4$	95°C(15 min); 32 cycles of [94°C(30s)+54°C(90 sec)+72°C(60s)]; 60°C(30min)	AY047223
Pca103	F: AGAAAGGTCAAGTGTATTAGCC R: CAAATACGAGTAAGTTCTTTAGTGC	$(ATT)_{15}(AGT)_4$	95°C(15 min); 32 cycles of [94°C(30s)+54°C(90 sec)+72°C(60s)]; 60°C(30min)	AY047221
Pca104B	F: GACACACATACACTTTCTCCATC R: GCTTGCTTCCTTGAGTGT	(TATC)	92°C(5 min); 32 cycles of [92°C(30s)+56°C(30 sec)+1°C/s to 72°C+72°C(20s)]; 72°C(30min)	Not yet submitted
Pca107	F: ACCTCTCGTTGTAACTGTGC R: TACACCTTGCTGTTCAGTCC	(CTAT)	92°C(5 min); 32 cycles of [92°C(30s)+56°C(30 sec)+1°C/s to 72°C+72°C(20s)]; 72°C(30min)	AY047227
Pca201	F: ACTTTCGCTTCTGGGGGCAG R: GGCATGCTTATAACGTGCAG	(CATA)	94°C(2 min); 32 cycles of [94°C(30s)+60°C(40 sec)+72°C(60s)]; 72°C(20min)	Not yet submitted

Reactions included forward primers fluorescently-labeled with IRDye® infrared dye (LI-COR, Lincoln, NE). Fluorescently labeled primers composed 10% of the total forward primer concentration in each PCR reaction. Microsatellite loci were amplified using a DNA Engine Tetrad PTC-225 Peltier thermal cycler (Bio-Rad Laboratories, Hercules, CA) and resulting amplicons were visualized on a LI-COR 4300 DNA Analyzer. Microsatellite allele sizes were determined using Saga Generation 2 microsatellite analysis software v.3.2.1 (LI-COR, Lincoln, NE).

Genetic Diversity and Population Genetic Structure

I analyzed collections of red king crab from 11 locations throughout Alaska (Figure 1). The software GENEPOP v.4 (Rousset, 2008) was used to perform pseudo-exact tests for departure from Hardy-Weinberg expectations at each locus (Guo and Thompson, 1992) and estimate gametic disequilibrium. The program FSTAT v.2.9.3.2 (Goudet, 1995) was used to estimate allele frequencies, observed (H_O) and expected (H_E) heterozygosity (Levene, 1949), F-statistics, pairwise F_{ST} values, genetic diversity measures, and to perform pairwise homogeneity tests. Homogeneity tests were performed between temporally spaced collections within locations and collections were pooled for subsequent spatial analyses if no significant difference in allele frequency was detected. Weir and Cockerham's (1984) θ was used for estimates of F_{ST} and 95% confidence intervals for multilocus F_{ST} estimates were established by bootstrap sampling across all loci (Goudet et al., 1996). Additionally, G'_{ST} (Hedrick, 2005) a genetic differentiation measure similar to F_{ST}, but corrected for subpopulation homozygosity,

was estimated as using the program GenoDive v.2.0 (Meirmans and Van Tienderen, 2004). Correcting for within-population diversity can be important when dealing with highly polymorphic markers such as microsatellites because the maximum values possible for traditional F_{ST} and related measures are constrained by within-population genetic diversity from multiple alleles (Meirmans and Hedrick, 2011). Pairwise and overall estimates of Jost's (2008) D were obtained with the program SMOGD (Crawford, 2010). Red king crab allozyme allele frequencies from Grant et al. (2011) were also used to estimate Jost's (2008) D and to provide a comparison of differentiation levels with microsatellite data. Where appropriate, significance levels were adjusted with a sequential Bonferroni correction (Rice, 1989).

Spatial population genetic structure of red king crab was investigated with Spatial Analysis of Molecular Variance (SAMOVA) using the program SAMOVA v.1.0, which conducts a simulated annealing approach to maximize the proportion of total variance attributed to differences among groups of populations (Dupanloup et al., 2002). This eliminates the need to determine group assignment *a priori* when testing genetic structure. The annealing process was repeated 100 times to ensure the validity of the assignment of populations into K different groups. The process was repeated with values of $K = 2$ to 11. Suggested population groupings for each value of K were then tested in Arlequin v.3.1 (Excoffier et al., 2005) and the significant grouping that maximized the proportion of variance attributed to differences among groups of populations (F_{CT}) was chosen as the best population grouping. Each AMOVA analysis was performed with Arlequin using 10,000 random permutations to test for statistical significance.

Population structure was also inferred using the Bayesian clustering method of Pritchard et al. (2000) as implemented in the program Structure v.2.3.3, under the admixture model with correlated allele frequencies. A burn-in period of 100,000 steps, followed by 100,000 MCMC iterations was used to test the likelihood that red king crab collections belonged to K populations. A range of K from one to eleven was explored, with three replicates at each value of K.

The statistical software R v.2.10.1 (R Core Development Team, 2009) was used to test for significant differences in genetic diversity, to examine patterns of isolation by distance, and to visualize similarities among sample collections through ordination. A Kruskal-Wallis test, which is a rank-randomization test, was used to test for significant differences in allelic richness (AR) and expected heterozygosity (H_S) among collections. Differences in genetic diversity among regions were tested with analysis of variance (ANOVA) and Tukey's HSD tests. Tests for isolation by distance were conducted with a Mantel test in the *ecodist* R package (Goslee and Urban, 2007). Ten thousand permutations of the data were performed and 10,000 bootstrap replicates were used to calculate 95% confidence intervals. Principal component analysis (PCA) was performed on arcsine-square root transformed allele frequencies with the *adegenet* R package (Jombart, 2008). Alleles with frequencies less than 0.05 in every sample were excluded to mitigate the effects of these rare alleles, while still maintaining most of the variation in the data. PCA is useful for visualizing relationships among multivariate data, because most of the variability in the data is captured in a small number of principal components without the necessity of adhering to strict and possibly unrealistic population structure

model assumptions. Linear discriminant analysis was then performed on the first 50 principal components as a way to maximize among-group variation while minimizing within-group variation (Jombart et al., 2010).

Effective Population Size and Population Bottlenecks

Single sample and temporal sample methods were used to estimate N_e. Single sample estimates were performed with the bias-corrected linkage disequilibrium method implemented in LDNE v.1.31 (Waples and Do, 2008). Estimates of N_e and migration rate (m) were obtained by examining changes in allele frequency for each of the six collections where temporally spaced samples were available, using pseudolikelihood-based estimation implemented in the program MNE v.2.0 (50,000 as the maximum value of N_e; Wang and Whitlock, 2003). Multiple estimates of N_e were obtained over a range of generation times (from one to three generations) to account for uncertainty in the true generation time of red king crab. Estimates of mean age at maturity for female red king crab are approximately 6-7 years, suggesting that the generation time is slightly higher than that. Great uncertainty exists in the estimates, however, due to difficulties in determining the age of a species that does not retain any calcified structures over molts (Zhou et al., 1998; Kittaka and Onda, 2002). The time between samples for the Pribilof Islands was 12 years while the time between samples for all other locations was 19 years (Figure 1). Joint estimation of N_e and m via the method of Wang and Whitlock (2003) requires specification of allele frequencies from a source population in addition to the temporal samples from the focal population. Since the specific source population of each

migrant is unknown, we pooled all remaining collections from two geographic regions (Southeast Alaska and all others) as the source population for each collection within that given region based on the results of Seeb et al. (2001).

The program BOTTLENECK v.1.2.02 (Cornuet and Luikart, 1996) was used to test for genetic signals resulting from severe decreases in abundance of red king crab in recent years. When a population experiences a severe bottleneck there is a loss of both heterozygosity and allelic diversity. For a period of time after a bottleneck event, the reduction in allelic diversity progresses more rapidly than the decrease in heterozygosity, resulting in heterozygosity that is greater than what would be expected for the same number of loci if the system were at mutation-drift equilibrium (Cornuet and Luikart, 1996; McEachern et al., 2011). A two-phase model (TPM), which is a combination of a stepwise mutation model (SMM) and an infinite alleles model (IAM) was used because it has been shown to fit observed allele frequencies for microsatellite data better than either of the other two individual models (Di Rienzo et al., 1994; McEachern et al., 2011). Three proportions of SMM in the TPM were used (70%, 80%, and 90% SMM) and excess heterozygosity was tested with a Wilcoxon signed-rank test. Populations were pooled as determined previously by homogeneity tests.

Mating System

Power to detect multiple paternity within a sample of embryos and Z1 from a single brood was determined with the program PrDM v.1 (Neff and Pitcher, 2002). The program PrDM uses a Monte Carlo simulation method to determine the probability that

multiple mating will be detected given the number of loci used, the number of alleles at each locus, allele frequencies of the population, and the number of potential sires and their relative reproductive contributions (Neff and Pitcher, 2002). Allele frequencies for Bristol Bay red king crab in 2008 were determined with the program FSTAT v.2.9.3.2 (Goudet, 1995). Three loci with relatively high degrees of polymorphism were selected and simulations were conducted that involved two potential sires with relative reproductive contributions of gametes to zygotes of 1:1 and 9:1. The probability of detecting multiple paternity is also dependent on the number of offspring sampled, so scenarios involving 10 and 20 offspring were tested for each of the above paternal contribution ratios.

Single paternity would be rejected if three or more non-maternal alleles were present within any given brood at a single locus. Requiring three non-maternal alleles as the condition for rejecting single paternity provides a conservative estimate of the minimum number of potential contributing sires by assuming that sires are heterozygous at each locus (McKeown and Shaw, 2008).

Results

Genetic Diversity

No significant difference in H_S ($P = 0.20$, $df = 10$) or AR ($P = 0.12$, $df = 10$) values were observed among collections by a Kruskal-Wallis test, although there is a geographic break in allelic richness and expected heterozygosity that shows a disconnect from the Western Alaska and Gulf of Alaska to Southeast Alaska (Figure 2). There was

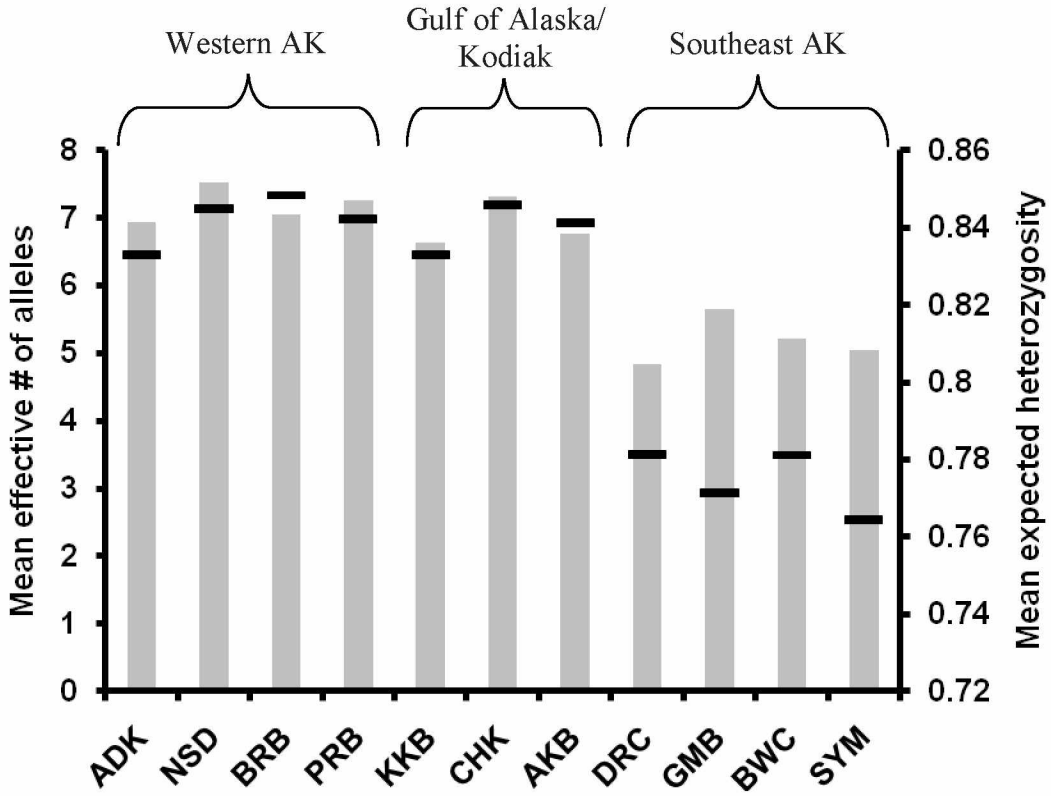

Figure 2. Mean effective number of alleles and mean expected heterozygosity. Mean effective number of alleles (columns) and mean expected heterozygosity (dashes) of red king crab populations. Locations: ADK (Adak Island), NSD (Norton Sound), BRB (Bristol Bay), PRB (Pribilof Islands), KKB (Kachemak Bay), CHK (Chiniak Bay), AKB (Alitak Bay), DRC (Deadman Reach), GMB (Gambier Bay), BWC (Barlow Cove), SYM (Seymour Canal).

a significant difference in H_S ($P < 0.001$, $df = 2$) and AR ($P = 0.001$, $df = 2$) among regions as determined by ANOVA. Tukey's HSD tests showed no difference between Western Alaska and Gulf of Alaska samples for H_S ($P = 0.93$) and AR ($P = 0.24$). Differences did exist between Southeast Alaska and the other two regions for both H_S (Western Alaska – Southeast Alaska, $P < 0.001$; Gulf of Alaska – Southeast Alaska, $P < 0.001$) and AR (Western Alaska – Southeast Alaska, $P = 0.008$; Gulf of Alaska – Southeast Alaska, $P = 0.001$). No populations showed departure from Hardy Weinberg expectations after sequential Bonferroni correction (initial $P > 0.05$). After sequential Bonferroni correction, no locus pair for a collection showed evidence of significant departure from gametic disequilibrium. Mean within-population expected heterozygosity (H_S) was 0.82 (range: 0.54 to 0.92) and mean within-population allelic richness (AR) was 9.62 (range: 5.24 to 14.63).

Temporal Population Structure

Temporal AMOVA of genetic variation over space and time suggested that temporal genetic variation (0.39%, $df = 6$, $P < 0.001$) accounted for less variation than was distributed spatially (2.03%, $df = 5$, $P < 0.001$), but both contributions were significant. Pairwise homogeneity tests were not significant within Norton Sound, Pribilof Islands, or Barlow Cove (Table 2), which suggested stability of allele frequencies over time. Collections for each of these three locations were pooled across years for

Table 2. Pairwise F_{ST} values and significance level of homogeneity tests for temporal collections. Pairwise F_{ST} values and significance level of homogeneity tests among pairs of temporally-spaced red king crab samples. Asterisks indicate significant results after correcting for multiple tests.

Sample	F_{ST}
Norton Sound	0.002
Bristol Bay	0.009*
Pribilof Islands	0.002
Deadman Reach	0.028*
Gambier Bay	0.013*
Barlow Cove	0.007

subsequent spatial analyses. Deadman Reach showed the most striking difference between temporal collections as indicated by the F_{ST} estimate (Table 2) and discriminant analysis of principal components (Figure 3). Temporally spaced collections within Barlow Cove and Gambier Bay in Southeast Alaska also showed a separation along the first and second discriminant axes, but no such relationship was immediately evident among temporally spaced collections in Western Alaska (Figure 3).

Spatial Population Structure

Moderate levels of population differentiation with an overall $F_{ST} = 0.025$ (SE = 0.009) were observed. After correction for multiple tests, 49 of 55 pairwise homogeneity tests were significant (Table 3). Pairwise comparisons between Southeast Alaska populations and all others were uniformly significant, as indicated by relatively high F_{ST} and D estimates and homogeneity tests (Table 3). Hedrick's (2005) correction for within-population genetic diversity, which is useful when dealing with highly polymorphic loci like those used in this study, provided evidence for greater divergence among populations with an overall $G'_{ST} = 0.128$ ($P = 0.001$, SE = 0.046). Jost's (2008) D provided an overall estimate of differentiation $D = 0.074$ (SE = 0.001). Overall $F_{ST} = 0.010$ (SE = 0.003) was observed among Adak Island, Norton Sound, Bristol Bay, and Pribilof Island collections in Western Alaska. Among Chiniak Bay, Alitak Bay, and Kachemak Bay collections in the Gulf of Alaska, overall $F_{ST} = 0.002$ (SE = 0.002) was observed. Overall differentiation was higher among Deadman Reach, Barlow Cove, Gambier Bay, and Seymour Canal collections in Southeast Alaska, with $F_{ST} = 0.021$ (SE = 0.013).

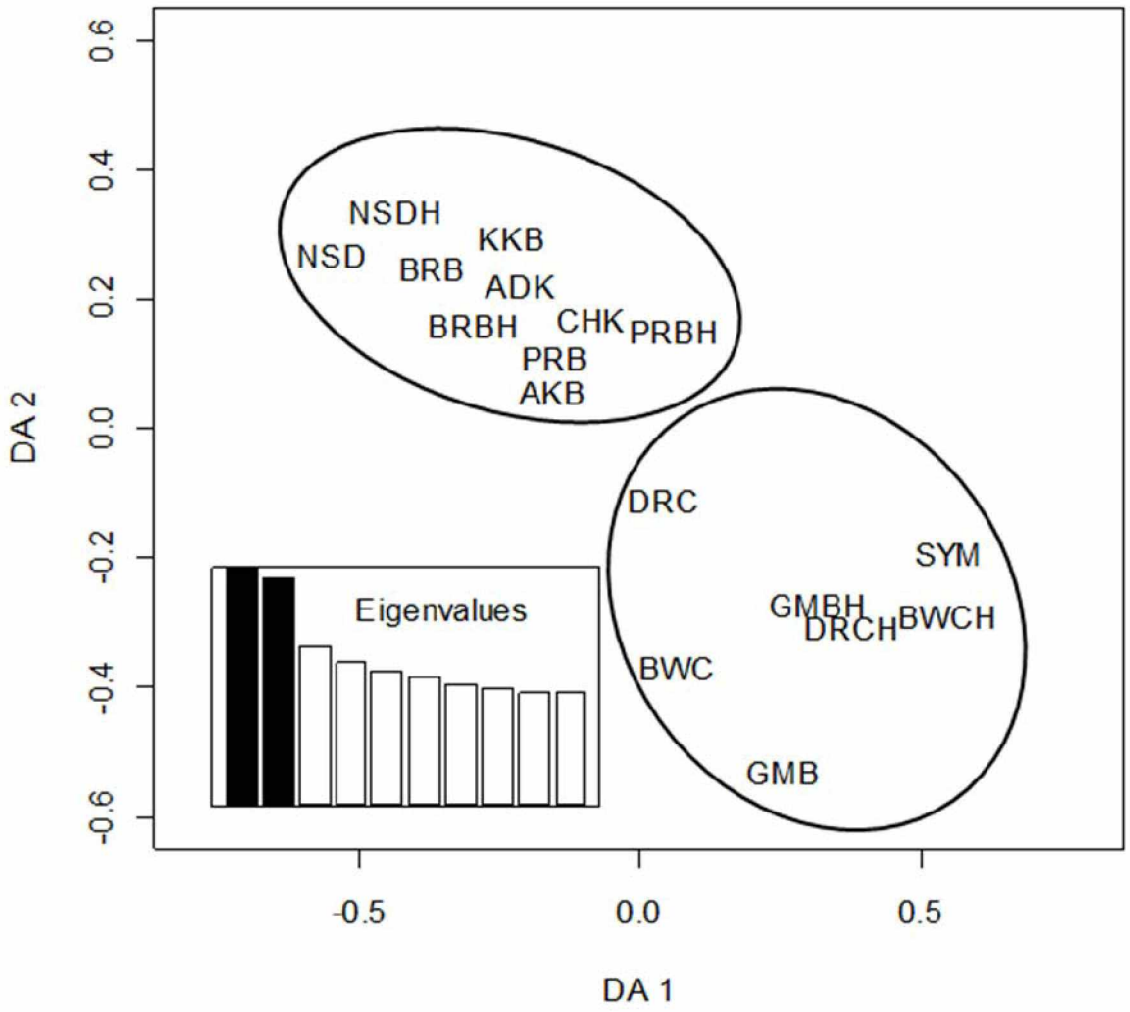

Figure 3. Linear discriminant analysis of principal components. Linear Discriminant analysis of principal components based on arcsine-square root transformed allele frequency data from 11 locations where red king crab were sampled. Ellipses represent the two regions of the Bering Sea/Gulf of Alaska, and Southeast Alaska. Locations: ADK (Adak Island), NSD (Norton Sound), BRB (Bristol Bay), PRB (Pribilof Islands), KKB (Kachemak Bay), CHK (Chiniak Bay), AKB (Alitak Bay), DRC (Deadman Reach), GMB (Gambier Bay), BWC (Barlow Cove), SYM (Seymour Canal). Four letter abbreviations ending in "H" denote historic samples. The inset graph shows the proportion of variation described by the first ten principal components.

Table 3. Pairwise F_{ST} values/D (Jost 2008) and significance values for homogeneity tests. Pairwise F_{ST} values/D (Jost 2008) are given below the diagonal and significance values for homogeneity tests are given on the upper diagonal. Asterisks indicate significance after sequential Bonferroni correction.

Populations	ADK	NSD	BRB	PRB	KKB	CHK	AKB	DRC	GMB	BWC	SYM
ADK	-	*	*	*	*	*	*	*	*	*	*
NSD	0.011/ 0.040	-	*	*	ns	*	*	*	*	*	*
BRB	0.016/ 0.054	0.007/ 0.015	-	*	*	*	*	*	*	*	*
PRB	0.007/ 0.027	0.010/ 0.022	0.014/ 0.038	-	*	ns	ns	*	*	*	*
KKB	0.015/ 0.067	0.004/ 0.014	0.014/ 0.060	0.006/ 0.013	-	ns	ns	*	*	*	*
CHK	0.014/ 0.047	0.008/ 0.025	0.008/ 0.021	0.003/ 0.001	0.004/ 0.003	-	ns	*	*	*	*
AKB	0.010/ 0.034	0.005/ 0.006	0.009/ 0.018	0.000/ 0.000	0.001/ 0.000	0.000/ 0.000	-	*	*	*	*
DRC	0.057/ 0.164	0.045/ 0.125	0.036/ 0.079	0.037/ 0.072	0.044/ 0.100	0.027/ 0.031	0.033/ 0.046	-	*	*	*
GMB	0.052/ 0.166	0.056/ 0.123	0.054/ 0.107	0.033/ 0.106	0.047/ 0.134	0.034/ 0.056	0.027/ 0.065	0.048/ 0.044	-	*	*
BWC	0.045/ 0.163	0.049/ 0.140	0.05/ 0.125	0.022/ 0.063	0.037/ 0.093	0.022/ 0.029	0.018/ 0.038	0.031/ 0.010	0.011/ 0.015	-	*
SYM	0.048/ 0.171	0.054/ 0.177	0.055/ 0.152	0.028/ 0.099	0.042/ 0.138	0.024/ 0.047	0.027/ 0.076	0.034/ 0.020	0.021/ 0.030	0.004/ 0.001	-

Locations: ADK (Adak Island), NSD (Norton Sound), BRB (Bristol Bay), PRB (Pribilof Islands), KKB (Kachemak Bay), CHK (Chiniak Bay), AKB (Alitak Bay), DRC (Deadman Reach), GMB (Gambier Bay), BWC (Barlow Cove), SYM (Seymour Canal).

Splitting all of the red king crab collections into five groups was determined by SAMOVA as the configuration that maximized the proportion of total variation attributed to differences among groups ($F_{CT} = 0.025$, $P < 0.001$; Table 4). Adak Island was the first group and the second group was composed of the remaining collections in the Western Alaska and Gulf of Alaska regions. Collections in Southeast Alaska were then divided into three groups, which included Gambier Bay and Deadman Reach as single-population groups, and Barlow Cove and Seymour Canal as a single group. Estimates of F_{CT} decreased for values of K above and below five, however estimates of F_{CT} were significant for values of $K = 3$ through 8 ($P < 0.05$; Table 4).

Bayesian clustering within the program Structure resulted in a maximum likelihood at $K = 2$. For values of K larger than two, the probability that samples came from that number of clusters decreased and the associated variance increased. The first cluster included the four collections from Southeast Alaska and the second cluster included all others (data not shown). Similarly, linear discriminant analysis of principal components of arcsine-square root transformed allele frequencies revealed a separation between Southeast Alaska collections and all others (Figure 3). The first principal component explained 42.3% of the total variation and the second principal component explained 11.7%. The first and second discriminant axes show a clear separation between Southeast Alaska collections and those in Western Alaska and the Gulf of Alaska (Figure 3). There is no distinct separation between Western Alaska and Gulf of Alaska collections. Discriminant analysis of principal components also shows greater

Table 4. Spatial analysis of molecular variance groupings. Spatial analysis of molecular variance (SAMOVA) groupings that maximized proportion of total variance among groups of populations (F_{CT}) and minimized values representing the extent of differentiation between populations within groups (F_{SC}) for $K = 2$ to 9.

K	Groupings	F_{SC}	F_{CT}	P
2	(ADK) and (all others)	0.022	0.002	0.542 ± 0.005
3	(ADK), (NSD, BRB, PRB, KKB, CHK, AKB), (DRC, GMB, BWC, SYM)	0.010	0.021	0.001 ± 0.000
4	(ADK), (NSD, BRB, PRB, KKB, CHK, AKB), (GMB), (DRC, BWC, SYM)	0.008	0.022	0.001 ± 0.000
5	(ADK), (NSD, BRB, PRB, KKB, CHK, AKB), (GMB), (DRC), (BWC, SYM)	0.005	0.025	0.000 ± 0.000
6	(ADK), (CHK), (NSD, BRB, PRB, KKB, AKB), (GMB), (DRC), (BWC, SYM)	0.005	0.022	0.001 ± 0.000
7	(ADK), (CHK), (NSD, BRB, PRB, KKB, AKB), (GMB), (DRC), (BWC), (SYM)	0.007	0.020	0.013 ± 0.001
8	(ADK), (CHK), (NSD), (BRB, PRB, KKB, AKB), (GMB), (DRC), (BWC), (SYM)	0.006	0.018	0.036 ± 0.002
9	(ADK), (CHK), (NSD), (BRB, ADKB), (PRB, KKB), (GMB), (DRC), (BWC), (SYM)	0.006	0.016	0.067 ± 0.003

Locations: ADK (Adak Island), NSD (Norton Sound), BRB (Bristol Bay), PRB (Pribilof Islands), KKB (Kachemak Bay), CHK (Chiniak Bay), AKB (Alitak Bay), DRC (Deadman Reach), GMB (Gambier Bay), BWC (Barlow Cove), SYM (Seymour Canal.

separation among Southeast Alaska collections than is seen for the remaining collections (Figure 3).

Comparison of pairwise F_{ST} /(1- F_{ST}) and straight-line distance matrices for all pairs of spatial populations (historic collections excluded) resulted in a significant isolation by distance pattern (Mantel $r = 0.618$, $df = 54$, $P < 0.001$). However, when populations were split into the two regions suggested by the other spatial analyses, the pattern broke down, for both Southeast Alaska (Mantel $r = -0.067$, $df = 5$, $P = 0.541$) and all other populations (Mantel $r = 0.52$, $df = 20$, $P = 0.064$). However, there is little power to detect a pattern within Southeast Alaska with only four collections.

Effective Population Size and Population Bottlenecks

Single sample estimates of N_e were negative for many collections (suggesting very large effective sizes) and all confidence intervals included infinity (Table 5). Conversely, estimates of N_e for all temporally collected samples were low (range: 24.77 to 117.20) and varied little from 1 to 3 generations (Table 6). Estimates of m were extremely high (range: 0.21 to 0.99) and varied more substantially than estimates of N_e over the range of possible generation times examined (Table 6).

Wilcoxon ranked-sign tests suggested recent population bottlenecks in several instances, depending on the underlying mutational model. Three collections had significant expected heterozygosity excess ($P < 0.05$) at 70% SMM (BRBH, DRC, and GMBH) and one collection at 80% SMM (BRBH). No collections had significant expected heterozygosity excess for the 90% SMM or 100% SMM models.

Table 5. Effective population size estimates and 95% confidence intervals from LDNe v.1.31. Effective population size (N_e) estimates and 95% confidence intervals calculated from the single sample estimator LDNe v.1.31.

Pop	N_e	U95%	L95%
ADK	533.7	Inf.	98.8
NSD pooled	1788.4	Inf.	225.2
NSD	-819.8	Inf.	183.0
NSDH	197.1	Inf.	61.6
BRB	-185.6	Inf.	158.9
BRBH	184.3	Inf.	74.6
PRB pooled	-761.6	Inf.	552.7
PRB	-360.8	Inf.	183.4
PRBH	-3121.4	Inf.	180.6
KKB	-293.0	Inf.	113.1
CHK	1863.8	Inf.	105.9
AKB	5770.0	Inf.	96.0
DRC	-794.3	Inf.	96.4
DRCH	995.9	Inf.	63.5
GMB	266.1	Inf.	66.4
GMBH	810.1	Inf.	67.3
BWC pooled	-492.2	Inf.	148.3
BWC	-112.2	Inf.	164.0
BWCH	177.9	Inf.	56.0
SYM	571.5	Inf.	89.6

Locations: ADK (Adak Island), NSD (Norton Sound), BRB (Bristol Bay), PRB (Pribilof Islands), KKB (Kachemak Bay), CHK (Chiniak Bay), AKB (Alitak Bay), DRC (Deadman Reach), GMB (Gambier Bay), BWC (Barlow Cove), SYM (Seymour Canal). Four letter abbreviations ending in "H" denote historic samples.

Table 6. Effective population size and migration rate estimates and 95% confidence intervals from MNE v.2.0. Effective population size (N_e) and migration rate (m) estimates and 95% confidence intervals calculated from the temporally spaced sample estimator MNE v.2.0. A range of generation times from one to three were used.

	$g = 1$		$g = 2$		$g = 3$	
Population	N_e	m	N_e	m	N_e	m
Norton Sound	91.56 (58.80-224.24)	0.61 (0.19-1.00)	103.44 (61.47-288.94)	0.41 (0.13-1.00)	117.2 (64.20-374.51)	0.33 (0.10-1.00)
Bristol Bay	74.16 (51.42-114.01)	0.94 (0.46-1.00)	77.21 (52.79-137.76)	0.59 (0.26-1.00)	84.52 (54.19-167.17)	0.46 (0.19-1.00)
Pribilof Islands	64.85 (43.93-107.51)	0.72 (0.36-1.00)	68.29 (44.79-129.03)	0.55 (0.25-1.00)	72.3 (45.63-152.55)	0.48 (0.19-1.00)
Deadman Reach	24.77 (19.73-32.09)	0.89 (0.43-1.00)	29.18 (22.36-41.21)	0.38 (0.22-0.61)	33.06 (24.09-48.22)	0.29 (0.17-0.47)
Gambier Bay	58.92 (37.19-148.42)	0.35 (0.13-0.82)	71.01 (41.94-165.92)	0.26 (0.10-0.57)	83.14 (46.61-199.95)	0.21 (0.08-0.48)
Barlow Cove	25.53 (20.71-32.58)	0.99 (0.88-1.00)	27.09 (21.33-34.70)	0.67 (0.44-1.00)	29.37 (22.62-40.18)	0.48 (0.31-0.73)

Mating System

The three microsatellite loci used in the study provided a high degree of confidence that multiple paternity would be detected, should it exist. Analyzing genotypes from 20 offspring per brood (the number assayed in the study) provided a probability of detecting multiple paternity of 99.8% when assuming equal contribution of gametes from two males to each brood and 85.6% when assuming an unequal input of gametes from two males to each brood at a ratio of 9:1. No evidence of multiple paternity was detected in 24 broods of red king crab, because each brood included two or fewer non-maternal alleles at each locus (Table 7). Each brood consisted of genotypes that could be produced by the known maternal genotype and the contribution of a single male of unknown genotype.

Discussion

Genetic Diversity and Population Structure

The most striking patterns in red king crab population genetic structure come from the differences seen in populations from Southeast Alaska versus those from the rest of Alaska. The populations in Southeast Alaska show the highest levels of within-region genetic divergence, as well as high genetic divergence from populations in the rest of the study area, while also having the lowest within-population genetic diversity. This may be a result of genetic drift acting at a faster rate on relatively smaller red king crab populations in Southeast Alaska or the results of deeper historic patterns such as re-colonization from glacial refugia. Southeast Alaska is dominated by small bay and fjord

Table 7. Alleles detected at each microsatellite locus within each brood as determined by genotyping offspring and mothers. Multiple paternity would be indicated by the presence of three or more non-maternal alleles at a given locus within a brood. Allele sizes represent the number of base pairs. Maternal alleles are in **bold**.

Year	Female and brood	*Pca101 alleles*	*Pca103 alleles*	*Pca107 alleles*
2007	1	**247**, **263**, 267	**245**, 248, **260**, 269	215, **223**, **231**, 235
	2	**247**, 263, **267**	**248**, 251, **257**	**223**, 227, **231**
	3	251, **255**, 259, **263**	**257**, **269**	235, **243**, **263**
	4	247, **251**, **263**, 279	**248**, 254, **257**	**211**, 223, 235, **239**
	5	239, **243**, 259, **263**	**248**, 251, **257**, 260	223, **235**, **259**
	6	243, 251, **259**, **263**	**251**, **257**, 269	239, 247, **259**, **263**
	7	223, **227**	**245**, **254**, 257	**203**, 211, 235
	8	**227**, 235	**257**, 266	**223**, 231, 247
	9	251, 255, **263**, **267**	248, **254**	235, **255**, **263**, 271
	10	243, **247**, **267**	**248**, **257**	**203**, **227**, 235, 259
	11	243, **251**	**248**, **254**, 257, 260	**235**, 239, **255**
	12	**239**, 243, 259, **263**	**245**, 248	231, **239**, **243**, 255
	13	**227**, 235, **263**	245, **254**	**223**, 243, **251**, 259
	14	**235**, 239, **251**, 267	**251**, **254**	235, **243**, **259**
	15	251, **259**, **263**	248, 251, **260**, **263**	**235**, 239, 243, **251**
	16	247, **255**, **263**	**248**, **257**	219, **251**, **255**
	17	**243**, **259**, 267	**245**, 254, **260**	**223**, **255**, 257, 263
	18	**223**, 239, **251**, 263	248, **257**, **263**	203, **227**, **235**, 271
	19	239, **243**, 247, **259**	251, **257**, **269**	**235**, **243**, 255
	20	243, 251, **259**	**248**, 251, **257**, 260	**223**, 243, **247**, 255
	21	239, **243**, **259**, 263	**248**, 251, 257	227, **239**, 263, **265**
	22	**243**, 247, **255**, 259	**251**, 254, **257**, 269	**223**, 235, 255, **259**
	23	237, **243**, 255, **263**	**245**, 251, **254**	223, **251**, 259, **271**
	24	**227**, 237, **243**, 263	**242**, 251, **254**	**235**, 239, **247**

systems (Weingartner et al., 2009) and localized currents in this region may result in higher degrees of local larval retention than are present in the more open waters of the Bering Sea and Western Alaska. Southeast Alaska is also near the southernmost range of red king crab and populations are unlikely to receive larval input from other distant locations due to the predominantly northward flow of the Alaska Current (Stabeno et al., 2004).

Results of temporal genetic structure analyses provide evidence for heterogeneity within some locations. Significant shifts in allele frequency over time observed within Bristol Bay, Deadman Reach, and Gambier Bay, may be the result of recent population bottlenecks. Bottlenecks occur when a population undergoes a drastic reduction in effective size. This leads to an increase in heterozygosity of sampled selectively neutral loci, compared to what would be expected for the same number of alleles if the population were at mutation-drift equilibrium. This condition may persist for several generations until a new equilibrium is reached (Cornuet and Luikart, 1996). A recent bottleneck in Deadman Reach is consistent with the observed change in allele frequency as well as reports from local biologists that red king crab in the area have been at extremely low density during recent stock abundance surveys (G.H. Bishop, Alaska Department of Fish and Game, Juneau, Alaska, personal communication, 2011).

Bottleneck signals observed in Bristol Bay, Deadman Reach, and Gambier Bay are also consistent with the results of Grant et al. (2011), who suggested bottlenecks in red king crab may be indicative of populations that have not yet reached mutation-drift equilibrium after demographic disturbance during the last ice-age. However, false

bottleneck signals have recently been observed among single populations within a stepping-stone or island model (Wakeley, 1999; Stadler et al., 2009). Chikhi et al. (2010) demonstrated that this effect is most prevalent when employing markers with high variability or when populations have a large genetic effective size. It is also worth noting that significant bottleneck signals observed in this study were also highly dependent on the assumed mutational model.

Estimates of N_e suggest large effective sizes in populations of red king crab in Alaska, but evidence of moderate population structure within broad geographic regions suggest some levels of gene flow and violate one of the basic assumptions of the single sample estimator of Waples and Do (2008) and likely accounts for the imprecision observed in the estimates and confidence intervals. Wang and Whitlock's (2003) method for joint estimation of N_e and m provided extremely low estimates of N_e and unrealistically high estimates of m. This same phenomenon was reported by Hoffman et al. (2004) and it was suggested that bias in these directions may be a result of uncertainty related to possible source population(s) of immigrants. Wang and Whitlock's (2003) method assumes an infinite source population and that source(s) of migrants be identified *a priori*. Moderate rates of gene flow in multiple directions within regions may explain the lack of reliable estimates we observed.

Overall levels of genetic divergence among collections suggest some moderate structuring of red king crab populations in Alaska. The overall F_{ST} estimate (0.025) for this study is between estimates reported in previous studies that used allozymes (F_{ST} = 0.003; Grant et al., 2011) and SNPs (F_{ST} = 0.045; Cheng et al., in press) over the same

approximate geographic range. The overall estimate of Jost's (2008) D for this study (0.074) was substantially higher than overall D (<0.001) estimated by averaging across allozyme loci using the data of Grant et al. (2011). Levels of differentiation observed in other invertebrate species with planktonic larval periods similar to red king crab were often lower than observed in this study. Puebla et al. (2008) reported F_{ST} = 0.011 (95% CI: 0.008-0.015) for snow crab (*Chionoecetes opilio*) in the northwest Atlantic. Merkouris et al. (1998) reported F_{ST} = 0.0046 for allozyme data from Tanner crab (*Chionoecetes bairdi*) in Alaska. Gaffney et al. (2010) reported F_{ST} = 0.004 (range: -0.005-0.0011) from microsatellite data for weathervane scallops (*Patinopecten caurinus*) in the northeast Pacific. Beacham et al. (2008) reported slightly higher overall differentiation (F_{ST} = 0.031, SD = 0.007) for Dungeness crab (*Cancer magister*) in British Columbia. Hedrick's (2005) measure of differentiation corrected for the high heterozygosity levels often present in microsatellite data and provided an overall measure of differentiation that is well above that provided by traditional F-statistics (F_{ST} = 0.025, G'_{ST} = 0.128). This number more accurately describe the absolute degree of differentiation among collections, because it adjusts for the maximum within-population expected heterozygosity, while simultaneously making it easier to compare levels of differentiation among studies that employ markers with differing levels of variability (Meirmans and Hedrick, 2011).

As expected based on the results of previous red king crab population genetic structure studies (Seeb et al., 1989; Seeb et al., 2001; Grant et al., 2011) and the large geographic disconnect between regions, the greatest degree of genetic differentiation

exists between populations in Southeast Alaska and all other locations. Results from discriminant analysis of principal components, Bayesian clustering analysis, and pairwise F_{ST} estimates support this two-region model of broad genetic differentiation. The large geographic distance between Southeast Alaska and the rest of the sample locations make the potential for larval transfer less likely. Complex currents within Southeast Alaska and the Gulf of Alaska may also inhibit larval transport between them, resulting in two regional metapopulations that are genetically distinct from one another.

Lower levels of among-population differentiation occur within the Western Alaska/Bering Sea region than in Southeast Alaska. Larger population sizes and increased connectivity in Western Alaska likely account for this decrease in genetic divergence among populations. Ocean currents flow predominantly from the western Gulf of Alaska through passes in the Aleutian Islands and northward into the Bering Sea (Stabeno et al., 2001; Stabeno et al., 2004). These currents may serve as a conduit for larval transport and provide a mechanism for connectivity, in contrast to Southeast Alaska where more complex oceanography may inhibit gene flow.

While the greatest differences exist between Southeast Alaska and the Western Alaska/Gulf of Alaska regions, SAMOVA results suggest some finer-scale genetic structuring within regions. The apparent difference between Adak Island and the other Western Alaska/Gulf of Alaska populations is likely due to the relative geographic isolation of Adak Island. Adak Island is located near the westernmost end of the Aleutian Islands and larval migration to and from the area is likely to be interrupted by the complex ocean currents that exist within the Aleutian Island chain (Stabeno et al., 2001;

Stabeno et al., 2004). In contrast, red king crab populations within Southeast Alaska show signs of genetic differentiation over a much smaller geographic scale. Smaller genetic effective sizes and decreased connectivity of populations in Southeast Alaska may account for this genetic structure.

Mating System

Genotypic evidence and the relatively high degree of confidence in the ability to detect multiple paternity based on the sample sizes and variability of loci used suggest female red king crabs are each mated by a single male. Single paternity is likely the dominant mating system in red king crab given the females' inability to store sperm and the mate-guarding behavior exhibited by male crabs. However, mating system may vary spatially and temporally for a given species (Sainte-Marie et al., 2002; Gosselin et al., 2005; Neff et al., 2008). Gosselin et al. (2005) suggested that spatial variation observed in the rates of multiple paternity for American lobster (*Homarus americanus*) may be a result of skewed sex ratios that result from sex-selective harvest of large males. Neff et al. (2008) reported that some of the variance observed in rates of multiple paternity of the guppy (*Poecilia reticulate*) could be explained by the levels of predation faced by different populations.

Our results suggest female red king crabs mate singly in a given year. This may make it easier to detect hatchery produced individuals in the wild from genetic tags. Fewer genetic markers may be employed to assign individuals to hatchery or wild origin if hatchery-reared family groups are less genetically diverse as a result of single paternity.

If enhancement of red king crab in Alaska takes place, these genetic markers can be used to monitor the survival, migration, and reproductive success of introduced individuals in order to determine the genetic impacts of the stocking program.

General Conclusions and Management Recommendations

Comprehension of the microevolutionary forces that influence population genetic structure over space and time is critical when dealing with species of commercial importance, such as red king crab in Alaska. Results of this study suggest moderate rates of gene flow within the Gulf of Alaska/Western Alaska region and support management of populations at a scale no smaller than that considered for this study. Levels of differentiation among populations within Southeast Alaska were slightly higher, and there was strong evidence of multiple distinct population groups, suggesting this region may warrant individual management decisions at a smaller geographic scale than is necessary elsewhere. Red king crab in Bristol Bay and the Deadman Reach and Gambier Bay areas in Southeast Alaska shows signs of recent population bottlenecks, not evident elsewhere, and may be indicative of overharvest and/or populations that have yet to reach mutation-drift equilibrium following the last ice-age. Single paternity in red king crab likely results in decreased genetic diversity within family groups and lower N_e. These factors will have to be considered if red king crab enhancement projects in Alaska ever take place.

References

Barber, P.H., Palumbi, S.R., Erdmann, M.V., Moosa, M.K., 2002, Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. Mol. Ecol. 11: 659-674.

Beacham, T.D., Supernault, J., Miller, K.M., 2008, Population structure of Dungeness crab (*Cancer magister*) in British Columbia. J. Shellfish Res. 27: 901-906.

Bechtol, W.R., Kruse, G.H., 2009, Reconstruction of historical abundance and recruitment of red king crab during 1960-2004 around Kodiak, Alaska. Fish Res. 100: 86-98.

Birkhead, T.R., Hunter, F.M., 1990, Mechanisms of sperm competition. Trends Ecol. Evol. 5: 48-52.

Bowers, F.R., Schwenzfeier, M., Coleman, S., Failor-Rounds, B., Milani, K., Herring, K., Salmon, M., Albert, M., 2008, Annual management report for the commercial and subsistence shellfish fisheries of the Aleutian Islands, Bering Sea and the Westward Region's Shellfish Observer Program, 2006/07 (Alaska Department of Fish and Game), p. 230.

Chapman, D.D., Prodohl, P.A., Gelsleichter, J., Manire, C.A., Shivji, M.S., 2004, Predominance of genetic monogamy by females in a hammerhead shark, *Sphyrna tiburo*: implications for shark conservation. Mol. Ecol. 13: 1965-1974.

Cheng, W., Guyon, J.R., Grant, W.S., Grauvogel, Z., Saski, C.A., Templin, W.D., in press, Characterization of 15 single nucleotide polymorphisms in Alaska red king crab. Mol. Ecol. Resour.

Chikhi, L., Sousa, V.C., Luisi, P., Goossens, B., Beaumont, M.A., 2010, The confounding effects of population structure, genetic diversity and the sampling scheme on the detection and quantification of population size changes. Genetics 186: 983-995.

Cornuet, J.M., Luikart, G., 1996, Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001-2014.

Crawford, N.G., 2010, SMOGD: software for the measurement of genetic diversity. Mol. Ecol. Resour. 10: 556-557.

Dew, C.B. 2011, Historical perspective on habitat essential to Bristol Bay red king crab, p. 377-402. In G. H. Kruse, G. L. Eckert, R. J. Foy, R. N. Lipcius, B. Sainte-Marie, D. L. Stram, and D. Woodby (editors), Biology and Management of Exploited Crab Populations under Climate Change. Alaska Sea Grant Program Report AK-SG-10-01, University of Alaska, Fairbanks, AK.

Dew, C.B., McConnaughey, R.A., 2005, Did trawling on the brood stock contribute to the collapse of Alaska's king crab? Ecol. Appl. 15: 919-941.

Di Rienzo, A., Peterson, A., Garza, J.C., Valdes, A.M., Slatkin M., Freimer, N.B., 1994, Mutational processes of simple-sequence repeat loci in human populations. Proc Natl. Acad. Sci. USA. 91: 3166–3170.

Dupanloup, I., Schneider, S., Excoffier, L., 2002, A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11: 2571-2581.

Excoffier, L., Laval, G., Schneider, S., 2005, Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol. Bioinform. 47-50.

Gaffney, P.M., Pascal, C.M., Barnhart, J., Grant, W.S., Seeb, J.E., 2010, Genetic homogeneity of weathervane scallops (*Patinopecten caurinus*) in the northeastern Pacific. Can. J. Fish. Aquat. Sci. 67: 1827-1839.

Goslee, S.C., Urban, D.L., 2007, The ecodist package for dissimilarity-based analysis of ecological data. J. Stat. Soft. 22: 1-19.

Gosselin, T., Sainte-Marie, B., Bernatchez, L., 2005, Geographic variation of multiple paternity in the American lobster, *Homarus americanus*. Mol. Ecol. 14: 1517-1525.

Goudet, J., 1995, FSTAT (Version 1.2): A computer program to calculate F-statistics. J. Hered. 86: 485-486.

Goudet, J., Raymond, M., De Meeus, T., Rousset, F., 1996, Testing differentiation in diploid populations. Genetics 144: 1933-1940.

Grant, W.S., Merkouris, S.E., Kruse, G.H., Seeb, L.W., 2011, Low allozyme heterozygosity in North Pacific and Bering Sea populations of red king crab (*Paralithodes camtschaticus*): adaptive specialization, population bottleneck, or metapopulation structure? ICES J. Mar. Sci. 68: 499-506.

Guo, S.W., Thompson, E.A., 1992, Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361-372.

Hauser, L., Carvalho, G.R., 2008, Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish. Fish. 9: 333-362.

Hedrick, P.W., 2005, A standardized genetic differentiation measure. Evolution 59: 1633-1638.

Hoffman, E.A., Schueler, F.W., Blouin, M.S., 2004, Effective population sizes and temporal stability of genetic structure in *Rana pipiens*, the northern leopard frog. Evolution 58: 2536-2545.

Ivanova, N.V., Dewaard, J.R., Hebert, P.D.N., 2006, An inexpensive, automation-friendly protocol for recovering high-quality DNA. Mol. Ecol. Notes 6: 998-1002.

Jombart, T., 2008, adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24: 1403-1405.

Jombart, T., Devillard, S., Balloux, F., 2010, Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Gen. 11: 94.

Jost, L., 2008, GST and its relatives do not measure differentiation. Mol. Ecol. 17: 4015-4026.

Kinlan, B.P., Gaines, S.D., Lester, S.E., 2005, Propagule dispersal and the scales of marine community process. Div. and Dist. 11: 139-148.

Kittaka, J., Onda, S., 2002, Effect of temperature on growth and maturity of the king crabs *Paralithodes brevipes* and *P. camtschaticus* in the laboratory. Fish. Sci. 68: 921-924.

Levene, H., 1949, On a matching problem arising in genetics. Ann. Math. Stat. 20: 91-94.

McEachern, M.B., Van Vuren, D.H., Floyd, C.H., May, B., Eadie, J.M., 2011, Bottlenecks and rescue effects in a fluctuating population of golden-mantled ground squirrels (*Spermophilus lateralis*). Conserv. Genet. 12: 285-296.

McKeown, N.J., Shaw, P.W., 2008, Single paternity within broods of the brown crab *Cancer pagurus*: a highly fecund species with long-term sperm storage. Mar. Ecol. Prog. Ser. 368: 209-215.

Meirmans, P.G., Hedrick, P.W., 2011, Assessing population structure: FST and related measures. Mol. Ecol. Resour. 11: 5-18.

Meirmans, P.G., Van Tienderen, P.H., 2004, GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4: 792-794.

Merkouris, S.E., Seeb, L.W., Murphy, M.C., 1998, Low levels of genetic diversity in highly exploited populations of Alaskan Tanner crabs, *Chionoecetes bairdi*, and Alaskan and Atlantic snow crabs, *C. opilio*. Fish. Bull. 96: 525-537.

Neff, B.D., Pitcher, T.E., 2002, Assessing the statistical power of genetic analyses to detect multiple mating in fishes. J. Fish Bio. 61: 79-750.

Neff, B.D., Pitcher, T.E., Ramnarine, I.W., 2008, Inter-population variation in multiple paternity and reproductive skew in the guppy. Mol. Ecol. 17: 2975-2984.

Orensanz, J.M.L., Armstrong, J., Armstrong, D., Hilborn, R., 1998, Crustacean resources are vulnerable to serial depletion - the multifaceted decline of crab and shrimp fisheries in the Greater Gulf of Alaska. Rev. Fish. Biol. Fish. 8: 117-176.

Otto, R.S., Jamieson, G.S., 2001, Commercially important crabs, shrimps and lobsters of the North Pacific Ocean (Sydney, B.C., Canada, PICES).

Palof, K., Heifetz, J., Gharrett, A., 2011, Geographic structure in Alaskan Pacific ocean perch (*Sebastes alutus*) indicates limited lifetime dispersal. Mar. Bio. 158: 779-792.

Palsboll, P.J., 1999, Genetic tagging: contemporary molecular ecology. Biol. J. Linnean Soc. 68: 3-22.

Palumbi, S.R., 1994, Genetic-divergence, reproductive isolation, and marine speciation. Ann. Rev. Ecol. and Syst. 25: 547-572.

Palumbi, S.R., 2004, Marine reserves and ocean neighborhoods: The spatial scale of marine populations and their management. Annu. Rev. Environ. Resour. 29: 31-68.

Powell, G.C., Nickerson, R.B., 1965, Reproduction of King Crabs, *Paralithodes camtschatica* (Tilesius). J. Fish. Res. Bd. Can. 22: 101-111.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000, Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Puebla, O., Sévigny, J.-M., Sainte-Marie, B., Brêthes, J.-C., Burmeister, A., Dawe, E.G., Moriyasu, M., 2008, Population genetic structure of the snow crab (*Chionoecetes opilio*) at the Northwest Atlantic scale. Can. J. Fish. Aquat. Res. 65: 425-436.

R Core Development Team, 2009, R: A language and environment for statistical computing (Vienna, Austria, R Foundation for Statistical Computing).

Rice, W.R., 1989, Analyzing tables of statistical tests. Evolution 43: 223-225.

Rousset, F., 2008, GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Resour. 8: 103-106.

Sainte-Marie, B., Sevigny, J.M., Carpentier, M., 2002, Interannual variability of sperm reserves and fecundity of primiparous females of the snow crab (*Chionoecetes opilio*) in relation to sex ratio. Can. J. Fish. Aquat. Sci. 59: 1932-1940.

Seeb, J., Kruse, G., Seeb, L., Weck, R., 1989, Genetic structure of red king crab populations in Alaska facilitates enforcement of fishing regulations. In: Proceedings of the International Symposium on King and Tanner Crabs, pp. 491-502.

Seeb, L.W., Grant, S., Templin, W.D., Kretschmer, E.J., Olsen, J.B., 2001, King and Tanner Crab Research in Alaska (Alaska Department of Fish and Game), pp. 14-28.

Seeb, L.W., Kretschmer, E.J., Olsen, J.B., Templin, W.D., Jones, K.C., Grant, W.S., 2002, Development of microsatellite loci in red king crab (*Paralithodes camtschaticus*). Mol. Ecol. Notes 2: 137-138.

Sherman, C.D.H., Hunt, A., Ayre, D.J., 2008, Is life history a barrier to dispersal? Contrasting patterns of genetic differentiation along an oceanographically complex coast. Biol. J. Linnean Soc. 95:106-116.

Stabeno, P.J., Bond, N.A., Hermann, A.J., Kachel, N.B., Mordy, C.W., Overland, J.E., 2004, Meteorology and oceanography of the Northern Gulf of Alaska. Cont. Shelf Res. 24: 859-897.

Stabeno, P.J., Bond, N.A., Kachel, N.B., Salo, S.A., Schumacher, J.D., 2001, On the temporal variability of the physical environment over the south-eastern Bering Sea. Fish Oceanogr. 10: 81-98.

Stadler, T., Haubold, B., Merino, C., Stephan, W., Pfaffelhuber, P., 2009, The impact of sampling schemes on the site frequency spectrum in nonequilibrium subdivided populations. Genetics 182: 205-216.

Strathmann, R.R., Hughes, T.R., Kuris, A.M., Lindeman, K.C., Morgan, S.G., Pandolfi, J.M., Warner, R.R., 2002, Evolution of local recruitment and its consequences for marine populations. Bull Mar. Sci. 70: 377-396.

Wakeley, J., 1999, Nonequilibrium migration in human history. Genetics 153: 1863-1871.

Wang, J.L., Whitlock, M.C., 2003, Estimating effective population size and migration rates from genetic samples over space and time. Genetics 163: 429-446.

Waples, R.S., 1998, Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. J. Hered. 89: 438-450.

Waples, R.S., Do, C., 2008, LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol. Ecol. Resour. 8: 753-756.

Ward, R.D., Woodwark, M., Skibinski, D.O.F., 1994, A comparison of genetic diversity levels in marine, fresh-water, and anadromous fishes. J. Fish. Bio. 44: 213-232.

Weingartner, T., Eisner, L., Eckert, G.L., Danielson, S., 2009, Southeast Alaska: oceanographic habitats and linkages. J. Biogeo. 36: 387-400.

Weir, B.S., Cockerham, C.C., 1984, Estimating F-statistics for the analysis of population-structure. Evolution 38: 1358-1370.

Wooster, W.S., 1992, King crab dethroned, In: Glantz, M.H. (Ed.) Climate Variability, Climate Change, and Fisheries. Cambridge University Press, New York, pp. 15-30.

Zhou, S.J., Shirley, T.C., Kruse, G.H., 1998, Feeding and growth of the red king crab *Paralithodes camtschaticus* under laboratory conditions. J. Crustac. Biol. 18: 337-345.

Appendix

Loadings for the first two principal components obtained from arcsine-square root transformed allele frequencies.

Locus	Allele	PC1	PC2
Pca100	280	0.1953	0.1127
Pca100	289	0.0018	-0.0791
Pca100	292	-0.1579	-0.0570
Pca100	295	0.0621	0.0823
Pca100	298	-0.1380	-0.0263
Pca100	301	-0.0332	-0.0211
Pca100	304	-0.1031	-0.1001
Pca101	227	-0.1441	0.0244
Pca101	235	-0.1257	0.1101
Pca101	239	-0.1635	0.0705
Pca101	243	-0.0835	0.2538
Pca101	247	-0.2725	-0.2598
Pca101	251	-0.1470	0.1881
Pca101	255	-0.0321	0.0322
Pca101	259	0.0339	-0.0522
Pca101	263	0.3839	0.2955
Pca101	267	0.0159	-0.5875
Pca103	242	-0.0027	0.0549
Pca103	245	0.0491	0.1880
Pca103	248	0.2257	-0.0755
Pca103	251	0.0331	0.0524
Pca103	254	0.0347	0.1064
Pca103	257	-0.2321	-0.0032
Pca103	260	-0.0375	-0.1006
Pca103	263	-0.0799	-0.1183
Pca103	266	-0.1981	-0.0051
Pca103	269	0.1337	-0.1031
Pca104	194	0.0404	0.1019
Pca104	202	-0.0030	-0.0398
Pca104	210	-0.0014	-0.0362
Pca104	214	-0.1088	0.0737
Pca104	222	-0.1668	0.1234

Appendix (continued)

Locus	Allele	PC1	PC2
Pca104	226	-0.1052	0.1295
Pca104	230	0.1408	-0.0696
Pca104	234	-0.1053	-0.0877
Pca104	238	-0.0227	-0.0261
Pca104	242	0.1827	-0.0559
Pca107	207	-0.1121	0.1183
Pca107	215	0.2139	0.0581
Pca107	223	0.0937	-0.0422
Pca107	227	0.0287	-0.0548
Pca107	235	-0.1177	-0.0142
Pca107	239	-0.1106	-0.0182
Pca107	243	0.0398	0.0158
Pca107	247	-0.0144	0.0226
Pca107	251	-0.0597	0.1428
Pca107	255	0.2257	-0.1251
Pca107	259	-0.1905	0.1853
Pca107	263	-0.1510	0.0732
Pca107	267	0.0096	-0.0197
Pca107	271	-0.0492	-0.0936
Pca201	318	0.0596	0.0660
Pca201	322	0.0167	-0.0272
Pca201	326	-0.0678	-0.0945
Pca201	330	0.0593	-0.0100
Pca201	334	-0.0656	0.0696
Pca201	338	0.1390	0.0390
Pca201	342	0.0740	-0.0567
Pca201	346	-0.1200	0.0166
Pca201	350	-0.1282	-0.0258
Pca201	354	-0.0858	0.1982
Pca201	362	-0.0593	0.0799