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Low allozyme heterozygosity in North Pacific and Bering Sea populations of red king crab (*Paralithodes camtschaticus*): adaptive specialization, population bottleneck, or metapopulation structure?

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Populations of red king crab in the North Pacific and Bering Sea have declined in response to ocean-climate shifts and to harvesting. An understanding of how populations are geographically structured is important to the management of these depressed resources. Here, the Mendelian variability at 38 enzyme-encoding loci was surveyed in 27 samples ($n = 2427$) from 18 general locations. Sample heterozygosities were low, averaging $H_E = 0.015$ among samples. Weak genetic structure was detected among three groups of populations, the Bering Sea, central Gulf of Alaska, and Southeast Alaska, but without significant isolation by distance among populations. A sample from Adak Island in the western Aleutians was genetically different from the remaining samples. The lack of differentiation among populations within regions may, in part, be due to post-glacial expansions and a lack of migration-drift equilibrium and to limited statistical power imposed by low levels of polymorphism. Departures from neutrality may reflect the effects of both selective and historical factors. The low allozyme diversity in red king crab may, in part, be attributable to adaptive specialization, background selection, ice-age population bottlenecks, or metapopulation dynamics in a climatically unstable North Pacific.

Keywords: background selection, marine crustacean, neutrality, Pleistocene, population bottleneck, population genetics.

Introduction

Mutation, migration, population size, and selection influence levels of genetic diversity within and among populations of marine species. Many benthic species potentially experience large amounts of gene flow among populations during planktonic larval stages. Gene flow tends to limit genetic divergence between populations (Waples, 1998) and produces geographically expansive populations with large effective population sizes. Large populations retain more genetic diversity than do small populations because the loss of genetic variability from genetic drift is less in large populations. Despite these predictions, several North Pacific decapods with planktonic larvae harbour lower levels of genetic diversity than do other marine species (Seeb *et al.*, 1990; Ward *et al.*, 1992; Merkouris *et al.*, 1998).

Several mechanisms may account for these reductions in the observed levels of genetic diversity. One is that effective population sizes (N_e) are not large because the potential for larval dispersal is not realized. Another mechanism producing low genetic diversity may stem from metapopulation dynamics, in which recurring local extinctions and founder effects during colonization erode genetic diversity (Smedbol *et al.*, 2002). Lastly, many ice ages have dominated the North Pacific over the past several thousand years and may have led to major population bottlenecks in many marine species. Insufficient time has elapsed since the last glacial maximum 18 000 years ago (Bond *et al.*, 1993) for genetic diversity to rebound through the accumulation of mutations (Nei *et al.*, 1975). Alternatively, background selection may prevent slightly deleterious new mutations from drifting to higher frequencies (Charlesworth *et al.*, 1993). Background selection, however, can produce allele-frequency distributions that are similar to those produced by population bottlenecks, making it difficult for researchers to distinguish among these hypotheses.

This study focuses on the red king crab (*Paralithodes camtschaticus*), which is the largest, most abundant, and most widespread of the species of king crab in the northeastern Pacific Ocean and Bering Sea. Native populations extend from the Sea of Japan in the northwestern Pacific (Sato, 1958) to British Columbia in the northeastern Pacific (Butler and Hart, 1962), and through the Bering Sea into the Chukchi Sea (Feder *et al.*,

2005). Several factors may influence the sizes and connectivity among populations of red king crab. Embryo dispersal is initially limited, because eggs stay attached to female pleopods for nearly a year before hatching (Marukawa, 1933). However, once larvae hatch, they can drift long distances in currents (Shirley and Shirley, 1989a). The patchiness of optimal habitats also influences population structure, because individuals at the semi-benthic post-larval stage (glaucothoe) actively choose suitable areas to settle (Stevens, 2003; Stevens and Swiney, 2005). Finally, adults often migrate several tens of kilometres in autumn each year to join large breeding congregations in shallow water (Dew, 1990; Stone *et al.*, 1992). The analysis of molecular markers may reveal the relative importance of these life-history influences on gene flow.

Abundances of most populations of red king crab in the Gulf of Alaska declined in the late 1960s, and fisheries throughout Alaskan waters were closed in the early 1980s (Kruse, 2007). Causes of these declines remain active areas of research, and the results of the studies indicate that combinations of overharvesting, trawling, and ocean-climate changes likely produced the declines (Dew and McConnaughey, 2005; Zheng and Kruse, 2006; Bechtol and Kruse, 2009). Fishery closures and limits on the total allowable catch have led to population recoveries in some areas (Kruse *et al.*, 2010), but not in others (Bechtol and Kruse, 2009). Uneven responses to these measures and ongoing research on population enhancement indicate a pressing need to consider population structure in the conservation and management of red king crab.

Several studies have previously surveyed genetic variability in the species. A survey of allozyme variability among eastern Bering Sea and Gulf of Alaska populations was used to define population groups for harvest management and enforcement (Seeb *et al.*, 1990). Although red king crab had low levels of genetic variability, allele frequencies partially resolved four major groups, including the eastern Bering Sea, Kodiak Island, Southeast Alaska, and a group that included crab from Adak Island, but also from the Gulf of Alaska. Another study of allozymes in a sample from Peter the Great Bay in the Sea of Japan also found low levels of genetic variability (Balakirev and Fedoseev, 2000). A recent study of microsatellite variability in samples from introduced populations in the Barents Sea found no drop in diversity in the introduced populations relative to samples from the Okhotsk and Bering Seas (Jørstad *et al.*, 2007). Lastly, another analysis of microsatellite variability among four Barents Sea samples and two northwestern Pacific samples detected little differentiation between the donor population and introduced Barents Sea populations and between Barents Sea populations (Zelenina *et al.*, 2008).

The chief motivation for this project was to search for genetic differences between populations that could be used for harvest management. The sample design of the study increased the number of allozyme loci surveyed and extended the number of samples examined by Seeb *et al.* (1990) from 13 to 18 locations, covering most of the species' range in North America. Although information on stock structure would enhance harvest management, the primary focus of the present study is on understanding the genetic architecture of red king crab. In particular, several hypotheses are considered to explain the relatively low levels of genetic variability in the species. These hypotheses include adaptive specialization, background selection, ice-age population bottlenecks, and metapopulation effects.

Material and methods

Sampling

In total, 2427 red king crabs were sampled from 1989 to 1996 at 18 locations (mean $n = 89.9$) extending from Norton Sound to southeastern Alaska and as far west as Adak Island (Figure 1 and Table 1). Crabs were collected by trawling or by pots during stock surveys, test fisheries, and commercial fisheries. Some

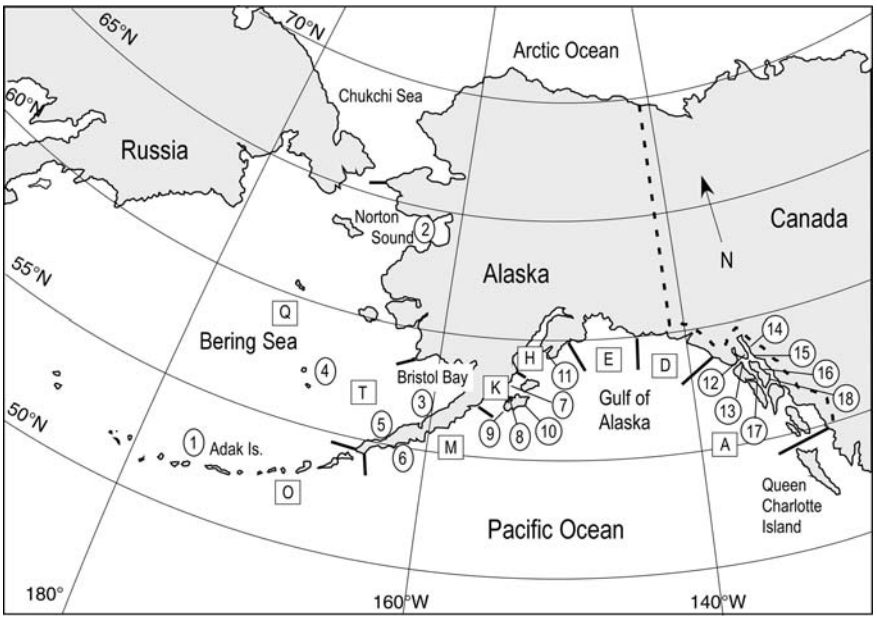

Figure 1. Map showing locations of the samples of red king crab in the Bering Sea and Gulf of Alaska. Locality numbers on the map correspond to locality numbers in Table 1, boxed letters indicate registration areas used to manage harvests of red king crab (Table 1), and bars along the coast indicate the extent of an area.

Table 1. Sample locations and summary statistics of allozyme analysis of red king crab.

Locality	Registration area	Date	Latitude	Longitude	*N*		H_O	H_E	P_{HD}
					Seeb *et al.*	This study			
Bering Sea									
1. Adak Island	O	1988	53°00′	175°34′	89	89	0.011	0.013	0.909***
2. Norton Sound	Q	1989	64°00′	165°28′	94	94	0.013	0.016	0.971***
3. Bristol Bay	T	1989–1993	56°57′–57°59′	160°20′–164°35′	6	381	0.013	0.015	0.917***
4. Pribilof Islands	T	1996	56°51′–57°25′	169°40′–170°31′	–	100	0.012	0.013	0.667
5. Port Moller	T	1990	56°04′	161°45′	–	51	0.018	0.014	0.789
Western Gulf of Alaska									
6. Pavlof Bay	M	1988	55°25′	161°35′	54	54	0.013	0.014	0.762
7. Kukak Bay	K	1991	58°18′	154°16′	–	62	0.015	0.014	0.857
8. Uganik Pass	K	1991	57°38′	153°13′	–	100	0.018	0.015	0.889***
9. Alitak Bay	K	1988	56°47′	154°16′	100	100	0.005	0.006	0.200
10. Chiniak Bay	K	1991	57°42′	152°25′	–	100	0.016	0.015	0.909
11. Kachemak Bay	H	1988	59°42′	151°11′	45	45	0.013	0.017	0.778
Southeast Alaska									
12. St James Bay	A	1988	58°05′	135°12′	94	94	0.012	0.013	0.800
13. Excursion Inlet	A	1988/1989	58°20′	135°28′	26	71	0.014	0.015	0.467
14. Eagle River	A	1988	58°30′	143°52′	100	100	0.011	0.012	0.913***
15. Barlow Cove	A	1988–1991	58°20′	134°53′	100	300	0.012	0.013	0.914***
16. Seymour Canal	A	1988–1993	57°49′	134°01′	100	199	0.010	0.013	0.967***
17. Deadman Reach	A	1988–1991	57°31′	135°30′	100	299	0.013	0.014	0.941***
18. Gambier Bay	A	1988/1989	57°27′	133°57′	100	100	0.011	0.013	0.800

N, number of crabs, including overlap with Seeb *et al.* (1990); H_O, observed heterozygosity; H_E, expected heterozygosity; P_{HD}, proportion of loci showing heterozygosity deficiency (distinct from the Hardy–Weinberg heterozygote deficit).
***$p < 0.001$.

small samples from trawling areas were pooled to increase the sample size. Multiple samples were collected in Bristol Bay (1989, 1991–1993), Barlow Cove (1988, 1990, 1991), Seymour Canal (1988–1989, 1993), and Deadman Reach (1988, 1989, 1991) to measure temporal heterogeneity in allele frequencies. Samples were collected from seven of the nine registration areas implemented by the State of Alaska for harvest management (Table 1, Figure 1). Portions of muscle, heart, gill, and hepatopancreas were placed in a tube on wet ice immediately after collection, then frozen shortly thereafter. Before 1991, samples were collected and shipped at −15°C, then stored at −80°C until electrophoretic analysis. After 1991, samples were immediately immersed in liquid nitrogen and stored at −80°C until analysis.

Allozyme electrophoresis

Enzymatic proteins in crude aqueous extracts from tissues were separated by starch-gel electrophoresis following the protocols in Seeb *et al.* (1990). Protein extracts from leg muscle, heart, gill, and hepatopancreas were separated with six electrophoretic buffer systems to resolve Mendelian banding patterns for products of 38 presumptive loci. Locus and allelic nomenclature followed Murphy *et al.* (1996).

Statistical analyses

Mendelian interpretations of electrophoretic banding variability were based on expected banding patterns for heterozygotes and homozygotes, given the subunit structure of an enzyme. The fit of genotypic frequencies to the Hardy–Weinberg expectations (HWEs) in each sample was tested with exact probability tests with a burn-in of 10 000 iterations, 20 batches of 10 000 iterations each (GENEPOP 4; Rousset, 2008). Probabilities over loci per sample and over loci and samples were determined with Fisher's method and with $p < 0.05$. Sample and locus heterozygosities were estimated with GENEPOP.

Temporal allele-frequency variability was examined among samples from Bristol Bay, Barlow Cove, Seymour Canal, and Deadman Reach with an exact *G*-test (GENEPOP), with burn-in of 10 000 steps, 100 batches, and 5000 steps for each batch. *G*-tests summed over loci did not detect significant temporal allele-frequency heterogeneity among four samples from Bristol Bay ($p = 0.183$), three samples from Barlow Cove ($p = 0.895$), two samples from Seymour Canal ($p = 0.725$), or three samples from Deadman Reach ($p = 0.128$). As no temporal heterogeneity was detected among samples at those sites, temporal samples were pooled into 18 groups for geographic analyses.

Geographic structure was examined with several statistics. Allele frequencies were compared among samples within and among regions with the exact *G*-test (GENEPOP). The number of migrants between populations each generation under the island model of migration was estimated using the frequencies and distributions of private alleles (GENEPOP). Overall population structure was estimated with unbiased *F*-statistics, as implemented in FSTAT 2.9 (Goudet, 2001). Isolation by distance was tested with Mantel's test and 10 000 permutations, as implemented in ISOLDE (Genepop on the web; http://genepop.curtin.edu.au/). Difference matrices for Mantel's test consisted of linearized F_{ST} and geographic distances (km) between samples.

Possible imprints of historical bottlenecks were examined with tests of drift-mutation disequilibria that can appear following a bottleneck in population size. Allele-frequency distributions were tested for fit to the Ewens–Watterson neutral sample distribution under the infinite-alleles model with BOTTLENECK 1.2.02 (Piry *et al.*, 1999). For these analyses,

samples were pooled by regions showing genetic homogeneity (Bering Sea, central Gulf of Alaska, and Southeast Alaska) and analysed separately. Allele frequencies were pooled into ten classes and tested for fit to the expected distribution with a one-tailed Wilcoxon test for heterozygosity deficiency and a rejection criterion of $p < 0.05$.

Results

Mendelian polymorphisms appeared in 16 of the 38 (42%) loci examined, but only a single locus, *Pgdh*, had a most common allele frequency overall <0.95 (Supplementary Table S1). *Ldh-3* and *G3pdh* had common allele frequencies <0.97 in at least one sample. Expected heterozygosities ranged from $H_E = 0.006$ to $H_E = 0.017$ among samples and averaged $H_E = 0.015$. Total heterozygosity in the total pooled sample was $H_E = 0.015$. No significant departure from HWE was detected over all samples with Fisher's probability ($p = 0.078$), although some of the tests for individual samples and loci were significant. Most of these significances were attributable to the sampling of low-frequency genotypes. However, the pooled sample from Bristol Bay showed positive values of F_{IS} and significant departures from HWE for *Ldh-3*, *sMdhA-2*, and *Pgdh*.

Excluding the sample from Adak Island, F_{ST} between samples ranged from −0.007 to 0.024 (Table 2). F_{ST} averaged 0.043 between the Adak Island sample and the remaining samples. Overall, bootstrapped $F_{ST} = 0.003$ (± 0.016) among the 18 samples, but an exact *G*-test indicated highly significant differences among populations over loci and for *Pgdh*. Samples were divided into three regions, Bering Sea (five samples), western and central Gulf of Alaska (five samples), and eastern Gulf of Alaska (eight samples) for additional tests. A *G*-test indicated highly significant allele-frequency differences among these three groups for *Pgdh*. No significant differences were detected among samples from the Gulf of Alaska (*Pgdh*, $p = 0.560$), nor among samples from Southeast Alaska (*Pgdh*, $p = 0.441$), but highly significant differences appeared among samples from the Bering Sea (*Pgdh*, $p = 0.0065$). Among the Bering Sea samples, the sample from Adak Island was a genetic and geographic outlier and was excluded. The new comparison among the three regions was still highly significant, but the samples within the Bering Sea were not significantly different ($p = 0.083$). Estimates of migration between populations using the frequencies of private alleles under the island model yielded 8.6 migrants between the 18 locations per generation. However, the number of migrants between populations within regions differed among regions. The model estimated the movement of 10.8 crabs between locations in the Bering Sea, 10.7 crabs between locations in the Gulf of Alaska, and 36.9 crabs in southeastern Alaska.

A test between $F_{ST}/(1 - F_{ST})$ and geographic distances between samples (Mantel's test, $p = 0.121$) indicated no isolation by distance among populations. However, divergences between the sample from Adak Island and other samples were somewhat larger than divergences between the other samples (Figure 2); hence, the test was repeated without the Adak Island sample. This test also failed to show a significant correlation between genetic and geographic distances ($p = 0.513$).

Allele-frequency distributions significantly deviated from neutral expectations in the three pooled samples representing the Bering Sea, central Gulf of Alaska, and Southeast Alaska. Each group showed a highly significant heterozygosity deficiency (Wilcoxon test, $p < 0.0003$). Allele-frequency distributions were

Table 2. Pairwise values of F_{ST} between samples of red king crab from 18 locations, sample numbers corresponding to those in Table 1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2	0.046																
3	0.055	−0.002															
4	0.019	0.004	0.007														
5	0.029	−0.005	−0.002	−0.005													
6	0.072	−0.003	0.001	0.020	0.007												
7	0.041	−0.005	−0.004	−0.001	−0.006	0.001											
8	0.044	−0.004	−0.002	0.002	−0.005	0.003	−0.006										
9	0.068	−0.005	−0.005	0.011	0.001	−0.009	−0.006	−0.004									
10	0.030	−0.001	0.002	−0.003	−0.006	0.012	−0.004	−0.003	0.004								
11	0.023	0.005	0.007	0.003	0.001	0.013	0.001	0.005	0.009	0.004							
12	0.013	0.007	0.010	−0.005	−0.004	0.024	0.001	0.004	0.015	−0.002	0.001						
13	0.037	−0.003	−0.002	−0.002	−0.006	0.004	−0.007	−0.004	−0.003	−0.004	0.000	−0.001					
14	0.033	−0.002	−0.000	−0.003	−0.007	0.009	−0.006	−0.004	0.001	−0.005	0.003	−0.002	−0.006				
15	0.068	0.001	−0.001	0.012	0.002	0.001	−0.002	−0.001	−0.006	0.005	0.014	0.016	−0.001	0.002			
16	0.052	−0.002	−0.002	0.004	−0.004	0.003	−0.005	−0.003	−0.005	−0.001	0.007	0.006	−0.005	−0.003	−0.001		
17	0.055	−0.001	−0.001	0.007	−0.002	0.003	−0.004	−0.002	−0.004	0.001	0.007	0.009	−0.003	−0.001	−0.001	−0.002	
18	0.047	−0.005	−0.003	0.001	−0.006	0.002	−0.007	−0.004	−0.005	−0.003	0.004	0.003	−0.006	−0.005	−0.002	−0.005	−0.003

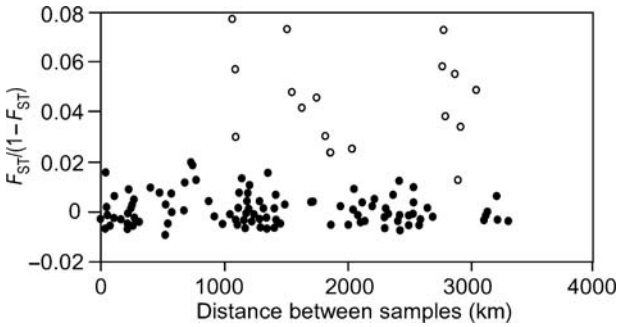

Figure 2. Scattergram of linearized F_{ST} and geographic distance between the samples of red king crab. Comparisons involving the sample from Adak are indicated by open circles.

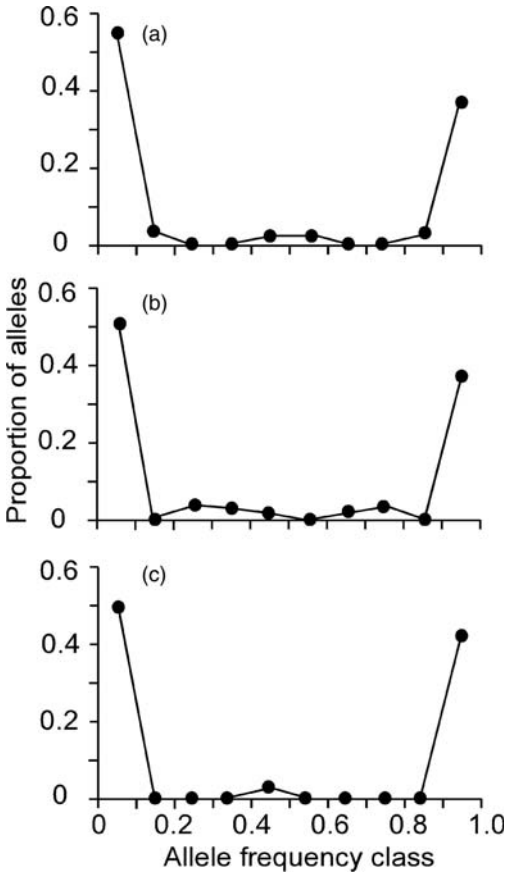

Figure 3. Allele-frequency distributions in three groups of red king crab from (a) the Bering Sea, (b) the northern Gulf of Alaska, and (c) southeastern Alaska.

L-shaped in each sample, with a large number of low-frequency alleles (Figure 3).

Discussion

Genetic population structure

The study was designed to search for genetic differences among populations of red king crab to facilitate the management of commercial harvests. Sample sizes were sufficiently large potentially to detect moderate allele-frequency differences between populations and between regional groups. The geographic distribution of samples used in the study included seven of the nine registration areas and reflected major areas of abundance. Unsampled registration areas have low abundances of red king crab. However, as most of the heterogeneity among samples was due to a single polymorphic locus, *Pgdh*, and because other loci showed only low-frequency minor alleles, the powers of tests for genetic differences between populations were low. Despite the low statistical power, allele frequencies differed significantly among samples overall and among three pooled groups, including the Bering Sea, Gulf of Alaska, and Southeast Alaska, in both Seeb *et al.* (1990) and the present study. No significant heterogeneity appeared among samples within these regions, except for the Bering Sea. The significant heterogeneity in the Bering Sea was attributable to differences between the Adak Island sample and the other samples. Nevertheless, genetic differences may exist at finer geographic scales that are not apparent with the analysis of the allozymes surveyed here. Further analysis with mitochondrial DNA (mtDNA) or nuclear DNA may reveal additional population structure.

Weak population structure is typical of marine species and is generally attributed to a large potential for gene flow through larval dispersal in currents. Although developing embryos of red king crab are retained in clutches by females for nearly a year, the dispersal of planktonic larvae upon release from the pleopods may be sufficient to produce genetic homogeneity among populations, at least over moderate distances. The results of this study could be useful to fishery management if these differences were to be interpreted under a gene-flow hypothesis. However, a pattern of isolation by distance was absent among populations, even in the presence of large-scale heterogeneity.

Several factors may prevent the appearance of isolation by distance in red king crab: complex coastlines, coastal currents, suitable habitats, and glacial history. Connectivity between populations is most likely mediated by larval drift in coastal currents, because adults are unlikely to move long distances. The Alaska Peninsula and the eastern end of the Aleutian Archipelago may act as barriers limiting the exchange of larvae between the Bering Sea and the Gulf of Alaska. Predominantly west-flowing coastal currents in the Gulf of Alaska may enhance the directional movement of larvae from eastern to western populations, creating a source–sink relationship among populations. However, the results do not support this model of population structure. Habitat patchiness in the eastern central Gulf of Alaska may also isolate populations, particularly if larvae do not move long distances. Lastly, historical isolations during ice ages could also produce genetic differences among populations.

The genetically distinct population groups may reflect isolations in Pleistocene glacial refugia. Marine refugia may have existed off the Queen Charlotte Islands (O'Reilly *et al.*, 1993), Kodiak Island (Karlstrom and Ball, 1969), and Beringia (Bickham *et al.*, 1995; Seeb and Crane, 1999). Other species in the North Pacific and North Atlantic have also not yet reached migration-drift equilibriums after post-glacial expansion from refugia (Carr and Marshall, 2008; Canino *et al.*, 2010; Grant *et al.*, 2010). Nevertheless, significant heterogeneity among regional groups of red king crab calls for separate management of populations on at least regional geographic scales. The use of smaller management units, as implemented by the State of Alaska, provides an even more conservative approach to the management of populations of red king crab.

Genetic diversity

A salient feature of this and other studies of red king crab is the low level of allozyme heterozygosity within populations. The overall estimate of heterozygosity for red king crab in the present study of North American populations was 1.5% (38 loci), similar to the low level of allozyme diversity (2.7%; 92 loci) reported in red king crab in the northwestern Pacific (Balakirev and Fedoseev, 2000). The difference between these two estimates may reflect geographic differences in the histories of Asian and North American populations or may be due to differences in the loci used to estimate heterozygosity. Balakirev and Fedoseev (2000) included loci not included in the present study. In particular, alkaline phosphatase appeared to be highly polymorphic in the Balakirev and Fedoseev (2000) study, but was not included here, because results for this locus were not always repeatable.

Allozyme heterozygosities are also small in commercially harvested Tanner crabs (*Chionoecetes bairdi*) in the North Pacific. Bering Sea populations of Tanner crab had an average observed heterozygosity of $H_O = 2.7\%$ (34 loci), and snow crab (*C. opilio*) had an observed heterozygosity of $H_O = 1.3\%$ (34 loci; Merkouris *et al.*, 1998). In red king crab and these two species of *Chionoecetes*, estimates of heterozygosity were somewhat smaller than heterozygosities in other marine invertebrates. In 105 species of molluscs, heterozygosity averaged 14.5% (Ward *et al.*, 1992). Even in crustaceans, which tend to have lower levels of genetic diversity than other invertebrates, heterozygosities averaged 5.2% among 80 species (Ward *et al.*, 1992).

What could have caused the low level of allozyme diversity in red king crab? One possibility is that these large crustaceans inhabit a fine-grain environment that promotes the fixation of a few advantageous alleles (Levins, 1968; Balakirev and Fedoseev, 2000). Restricted temperature tolerance of the various life-history stages has apparently limited the geographic distributions of lithodid king crab lineages to the North Pacific (Shirley and Shirley, 1989b; Hall and Thatje, 2009). Temperature is the environmental variable that most influences the efficiency of the metabolic enzymes used in allozyme studies. Selection against slightly deleterious alleles that prevents them from reaching high frequencies (background selection) may also limit allozyme diversity (Charlesworth *et al.*, 1993). The higher levels of diversity in microsatellite loci (Jørstad *et al.*, 2007; Zelenina *et al.*, 2008), which are assumed to be neutral, support this hypothesis. Even so, microsatellite variability in red king crab is not as high as it is in other marine fish, indicating that other mechanisms may limit allozyme diversity.

Metapopulation dynamics can also lead to low gene diversity. In this scenario, genetic diversity is lost when a local population is extirpated by climate shifts that influence temperature and foodweb dynamics, but is replenished locally by migration and colonization. A history of extinction and recolonization, however, can lead to a reduction in diversity, because colonists ultimately originate from populations that have experienced a loss of diversity (Hedrick and Gilpin, 1997). These metapopulation effects likely play out against a background of decadal and millennial ocean-climate cycles (Zheng and Kruse, 2000; Mantua and Hare, 2002).

In part, low allozyme diversities may also be due to population disturbances during the ice ages. Ice-age maxima produced latitudinal shifts in temperature and drops in the sea level that altered shoreline configurations and ocean current patterns (Hopkins, 1972). These environmental changes led to lower levels of productivity (Zahn *et al.*, 1991) and interacted with critical temperature thresholds of the various life-history stages of red king crab (Hall and Thatje, 2009). The physiological stresses likely led to local recruitment failures and shifts in distribution (Zheng and Kruse, 2000).

Reconstructions of palaeoclimatic conditions in the North Pacific indicate that many populations of red king crab may have been driven to extinction. Contemporary populations now inhabit many areas that were dry land during glacial maxima, or coastal areas that were covered with lobes of the Cordilleran ice sheet (Ager, 2003). Red king crab most likely survived these glacial maxima in offshore or southern refugia, as is apparent for many other North Pacific invertebrates (Barrie and Conway, 1999; Marko, 2004).

Tests for fit to the Ewens–Watterson neutral sampling distribution indicated significant departures from neutrality in red king crab, consistent with an ice-age bottleneck hypothesis. These departures were caused by an excess of low-frequency alleles, which produced a heterozygosity deficiency relative to the number of alleles in the samples. Departures from neutrality may be attributable to a selective regime such as background selection or may appear before a population reaches mutation-drift equilibrium following a severe bottleneck in population size. Immediately after a bottleneck, deficits of low-frequency mutations (heterozygosity excess, distinguished from the Hardy–Weinberg heterozygote excess) appear relative to equilibrium populations with the same level of genetic diversity (Nei *et al.*, 1975; Luikart and Cornuet, 1998). As population sizes recover, new mutations accumulate and produce excesses of low-frequency alleles (heterozygosity deficiency) for $\sim 0.4\, N_e$ generations (Maruyama and Fuerst, 1984), where N_e is the effective population size. Large populations experience a proportionally greater loss of alleles during a bottleneck (Ryman *et al.*, 1995) and a greater excess of low-frequency alleles during recovery (Maruyama and Fuerst, 1984). The mutation-frequency spectrum asymptotically approaches drift-mutation equilibrium after $\sim 2\, N$ generations, as low-frequency mutations drift to intermediate frequencies.

Conclusions

The low allozyme diversity in red king crab may result from a combination of several mechanisms. Selective forces may limit genetic diversity because of a narrowly adapted genome, or because of background selection. The North Pacific, however, has experienced many climate shifts on scales ranging from decades to millennia to hundreds of thousands of years. Major ice ages occur every 100 000 years, and they may have inflicted major bottlenecks on the populations sizes of red king crab and eroded genetic diversity. If additional analyses of microsatellites and mtDNA variability also show departures from neutrality, they would add weight to a population bottleneck hypothesis. Additionally, shorter-term climate cycles on decadal and millennial time-scales may also contribute to metapopulation extinctions and colonizations of this narrowly adapted crustacean. Such cycles tend to erode genetic diversity. Commercial harvests may further stress populations, as evidenced by population declines and harvest closures in some areas.

Supplementary material

Supplementary material is available at *ICESJMS* online, in the form of allele frequencies in the samples of red king crab.

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