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EXPERIMENTAL ARTICLES

Red King Crab (*Paralithodes camtschaticus*) in the Barents Sea: A Comparative Study of Introduced and Native Populations

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Abstract—Red king crab (*Paralithodes camtschaticus*) was introduced into the Barents Sea in the 1960–1970s. At present, it occurs along the coast from Hammerfest (Northern Norway) to the (northeastern coast of Kola Peninsula). We studied the polymorphism of a mitochondrial gene encoding cytochrome oxidase (*COI*) and five nuclear microsatellite loci in four samples from the Barents Sea and two donor populations from the Western Kamchatka and Primorye (Russian Pacifics). No decrease in the genetic diversity of the introduced populations was observed.The results of *PCA103* locusanalysis but some samples from the Barents Sea significantly differed from the Pacific populations according to the test for population differentiation demonstrated that the sample from Varrangerfjord was highly significantly different from the other five populations. As no significant differences between the other samples were found at this locus. We consider it to be a marker for Varangerfjord population differentiation. Possible reasons of this phenomenon are discussed.

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INTRODUCTION

The fauna of decapod crustaceans of the Barents Sea yields considerably in its genetic diversity to the Far Eastern seas. This includes the diversity of the family Lithodidae (Samouelle, 1819), or the so-called craboids. Until recently, only one species belonging to this family, the deep sea king crab *Lithodes maja* (Linnaeus, 1758) inhabited the Northeast Atlantic and the adjacent Arctic regions. The idea to increase the diversity of commercial crustaceous species in the Barents Sea dates back to the 1920s.

Starting from the 1930s, artificial introduction of some species belonging to this family from the Far Eastern seas has been repeatedly attempted. Red king crab *Paralithodes camtschaticus* (Tilesius, 1815), the main commercial species of the Far East, was selected as the object of these experiments. However, the experiments were unsuccessful until the systematic activities on acclimatization of red king crab were launched in 1961–1969 [1–3].

These activities included the release of about 3800 adult crabs, 10700 young individuals, and 1500000 larvae in the Barents Sea [4]. After the first findings of adult individuals in 1974 [5], this species became a regular dweller of the Murmansk Coast of the Barents Sea. In 1977, red king crab was for the first time found in the Norwegian waters [6]. Although there were grounds by that time to speak about a self-sustained population, mature and young individuals from the natural range were additionally introduced in 1977–1978.

In the majority of cases, the crabs for further introduction were captured in the Peter the Great Bay (Sea of Japan) and only one batch originated from the Sea of Okhotsk (western Kamchatka). The crabs shipped from the Far East were released in the Kola and Motovsky Bays and the adjacent regions of the Russian part of the Barents Sea. The present habitation area of red king crab spans from Hammerfest in the west to the Kolguev Island in the east and northern part of the White Sea Voronka (66°18′N) in the south [7] to about 72°N in the north [4]. The population of Barents Sea only in the Russian waters in 2003–2005 was estimated as at least 100 million individuals with about 40–50 million mature animals [8].

The precise data about the time when the population appeared and the number of introduced individuals as well as the reliable data on the donor populations give a unique possibility to study the processes underlying the establishment of the population structure of this long-lived representative of decapod crustaceans under new environmental conditions and its comparison with the parental populations.

Genetic studies of the red king crab from the natural populations of southern Primorye [9], Alaska coast [10], and western Kamchatka [11] have demonstrated a low level of intrapopulation allozyme variation, suggesting that this marker type is inappropriate for studying the population structure. Of all the markers used in population genetics, the best for this purpose are microsatellites, neutral and, as a rule, highly polymorphic markers with a high mutation rate, which are optimal for studying the evolutionary processes in closely related populations. The results of comparative studies of microsatellite polymorphism in two Pacific red king

Table 1. Characterization of the material studied

Sampling locality	Sampling date	Sample size	Designation
Barents Sea, Varrangerfjord	June 2003	53	V
Barents Sea, Dolgaya Bay	July 2003	42	Dl
Barents Sea, Teriberka Bay	June 2003	73	T
Barents Sea, Drozdovka Bay	May 2005	38	D
Western Kamchatka	November 2005	49	K
Peter the Great Bay	June 2006	60	Pr

crab populations and one Barents Sea population were recently published [12].

This paper reports the studies of the population structure of the red king crab introduced into the Barents Sea as compared with the donor populations from its natural habitat. Samples from four localities in the Barents Sea, western Kamchatka, and Peter the Great Bay were studied by assaying the polymorphism of mitochondrial gene encoding cytochrome oxidase (*COI*) and five nuclear microsatellite loci.

MATERIAL AND METHODS

Muscle tissue specimens of red king crab were sampled in 2003–2006. The sampling in the Barents Sea was conducted during an integrated diving survey in the littoral region of the Kola Peninsula; the sampling in the Seas of Japan and Okhotsk was performed with the help of commercial crab traps.

The sites with diverse conditions—inlets, bays, and open coast regions—were selected for diving surveys. At the selected sites, transects were set up perpendicular to the shoreline from the water edge to a depth of 30–45 m to capture red king crabs of various ages. The maximal carapax width of the caught crabs was measured and their sex was determined. A piece of muscle tissue (2–5 g) was cut off from the merus of the fourth right walking leg with a scalpel and forceps and fixed in 96% ethanol. In the Drozdovka Inlet, crab hatchlings with a length of 5–25 mm (weight, 1–7 g) were also caught and fixed as a whole.

Table 1 lists the characteristics and sizes of the samples studied.

Fig. 1 shows the sampling sites in the Barents Sea and the sites where red king crabs were released during their introduction.

Total DNA was isolated by salt extraction [13] from the muscle tissue fixed with 96% ethanol. DNA concentration and degree of purification were determined in a SPECTRAmax PLUS^{384} (Molecular Devices) plate spectrophotometer; the measurement results were calculated using the SOFTmax PRO (Molecular Devices) software. For microsatellite assay, the DNA concentrations were adjusted to 50 ng/μl to avoid a template overload.

Microsatellite loci were amplified using species-specific primers in an MJ Research PTC-225 Thermocycler. Table 2 lists characteristics of the primers used. The 5'-ends of forward primers were modified with a fluorescent dye.

PCR mixtures (15 μl) contained about 100 ng DNA, 70 mM Tris–HCl pH 8.3, 16.6 mM $(NH_4)_2SO_4$, 2 or 3 mM $MgCl_2$ (see Table 2), 100 mM of each deoxyribonucleoside triphosphate, 1.5 pM of each labeled primer, 3 pM of each unlabeled primer, and 1 U of Hot-*Taq* polymerase. The locus *Pca104* was amplified in the same reaction with *Pca107* (PCR-1) using the following scheme: melting at 95°C for 10 min; 30 cycles of melting at 94°C for 30 s, primer annealing at 56°C for 15 s, and DNA synthesis at 72°C for 15 s; and the final stage at 72°C for 10 min. PCR-2 (loci *Pca100* and *Pca101*) and PCR-3 (locus *Pca103*) was conducted according to a TouchDown scheme, namely, initial melting at 95°C for 15 min; 13 cycles of melting at 94°C for 5 s, primer annealing at an initial temperature of 64°C with a decrease by 0.5°C at each next step for 15 s, and DNA synthesis at 70°C for 20 s; 26 cycles of 90°C for 5 s, 58°C for 15 s, and 70°C for 20 s; and final synthesis at 72°C for 5 min.

Analysis of PCR products of microsatellite loci by capillary electrophoresis. PCR products were separated in a SpectruMedix SCE 9610 Genetic Analysis System for capillary electrophoresis for 1 h at a current stabilization of 2.3 μA per capillary. Before electrokinetic injection of analyzed DNA, each specimen was supplemented with HiDi formamide (10 μl per 3 μl of PCR product) and denatured at 96°C for 2 min. The marker modified with the fluorescent dye ROX was used as an internal standard. The lengths of alleles were determined using the Genospectrum Software (SpectruMedix LLC). Chromatographic pattern of a sample is shown in Fig. 2.

Sequencing of mtDNA COI fragment. The nucleotide sequence of a COI fragment (664 nucleotides) was determined for a random sample of 40 individuals from four populations and its polymorphism was studied. The universal primers LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) [15] were used for amplification conducted according to the following scheme: initial melting at 94° for 15 min; 37 cycles of melting at 94°C for 30 s, primer annealing

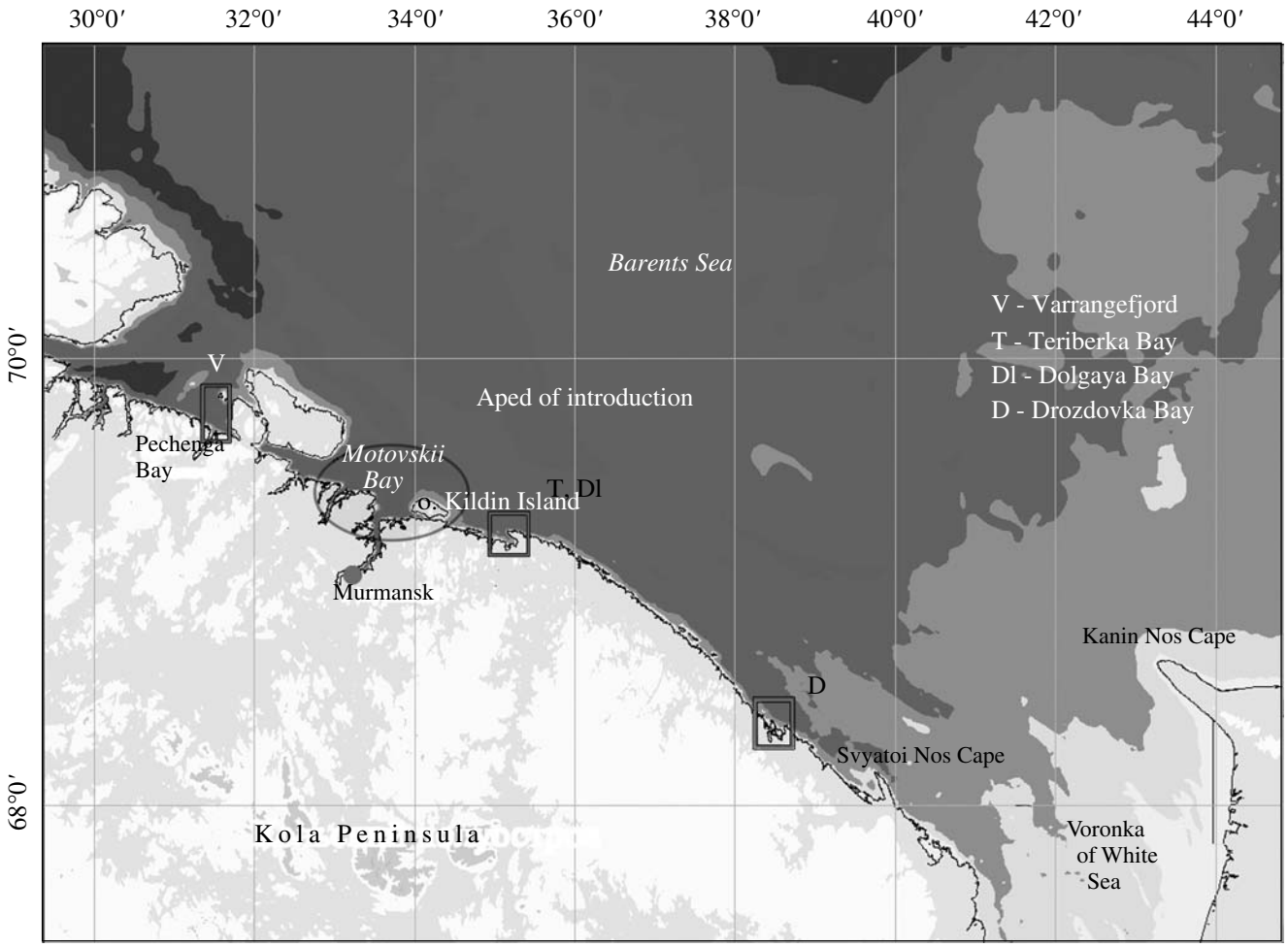

Fig. 1. Sampling sites (rectangles) in the Barents Sea and introduction regions (oval).

at 48°C for 1 min 30s, and DNA synthesis at 72°C for 1 min; and final synthesis at 72°C for 10 min.

Sequencing was conducted using the same primers in both directions in an ABI Prizm 3100 with a BigDye v.1.1 followed by sequence analysis and alignment using the LaserGene 6.0 software package.

The GENEPOP 3.4 software package [16] was used for statistical analysis of the data. The similarity between mitochondrial haplotypes was analyzed by maximum parsimony (MP) method; the minimal distance tree was constructed using the TSC program [17].

RESULTS

Analysis of Mitochondrial DNA Polymorphism

Figure. 3 shows the results of fragment length polymorphism analysis of the mitochondrial gene *COI*. Note that a large number of identical haplotypes were distributed among the individuals from different populations. Such picture along with the absence of clustering according to populations suggests the absence of differentiation in mitochondrial DNA not only among the samples from the Barents Sea, but also between the native populations.

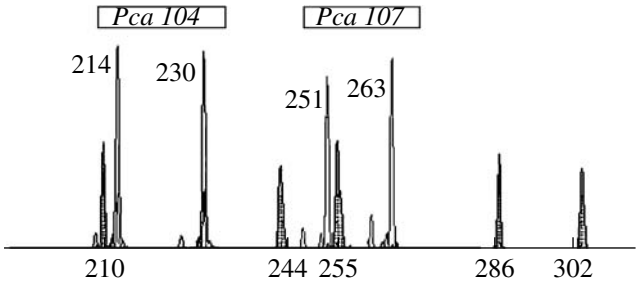

Fig. 2. An example of chromatogram of the duplex PCR products of one crab obtained in a 96-capillary SpectruMedix SCE 9610 Genetic Analysis System. This individual was heterozygous at the loci *Pca104* (lengths of alleles, 214 and 230 bp) and *Pca107* (251 and 263 bp).

Table 2. The primers used for microsatellite analysis (nucleotide sequences and repeated motifs of microsatellite loci are given according to [14])

Locus	Primer sequences	Repeated motif	Mg^{2+} concentration, mM	PCR variant
Pca100	Forward: 5'-FAM- GGTGCTCATCATTACTCAGG-3' Reverse: 5'-ACAGAGAAACGGATGAAGG-3'	$(TAA)_{11}$	3.0	PCR-2
Pca101	Forward: 5'-R6G- TTTCGGTTACTCGATATAATGC-3' Reverse: 5'-TTTTTCTCTGCTTACGAAGG-3'	$(TATC)_{18}AA$ $(TCAA)_4$	3.0	PCR-2
Pca103	Forward: 5'-TAMRA- AGAAAGGTCAAGTGTATTAGCC-3' Reverse: 5'-CAACTCCGAGTAAGTTCTTTAGTGC-3'	$(ATT)_{15}$ $(AGT)_4$	3.0	PCR-3
Pca104	Forward: 5'-R6G- ACAGACACACATACACTTTCTCC-3' Reverse: 5'-GTGGGATAACCATGACACC-3'	$(TATC)_2N_6$ $(TATC)_{10}$	2.0	PCR-1
Pca107	Forward: 5'-TAMRA- ACCTCTCGTTGTAACTGTGC-3' Reverse: 5'-TACACCTTGCTGTTCAGTCC-3'	$(CTAT)_{11}TT$ $(CTAT)_6N_7$ $(CTAT)_4N_{14}$ $(CTAT)_{12}AT$ $(CTAT)_3$	2.0	PCR-1

Table 3. Characterization of the allelic diversity of samples

Sample	Number of alleles per locus					
	Pca100	*Pca101*	*Pca103*	*Pca104*	*Pca107*	Mean
Barents Sea, Varrangerfjord	6	12	9	12	16	11
Barents Sea, Dolgaya Bay	6	14	10	13	12	11
Barents Sea, Teriberka Bay	6	15	12	13	16	12.4
Barents Sea, Drozdovka Bay	6	12	8	10	14	10
Western Kamchatka	*6*	*15*	*9*	*11*	*15*	*11.2*
Peter the Great Bay	*7*	*12*	*9*	*14*	*20*	*12.4*
Total	8	16	13	16	22	15

Note: Donor populations are italicized.

Microsatellite Analysis

The majority of the samples analyzed were at Hardy–Weinberg equilibrium at each locus; no linkage between the loci was detected. The numbers of alleles found for each locus are listed in Table 3.

No changes in the level of genetic polymorphism in the introduced populations compared with the donor populations from the natural habitat were detected (Table 3).

Allelic frequency distribution for each of the five loci is shown in Fig. 4.

The pairwise sample indices F_{st} failed to detect any significant differences between both the samples from various local sites in the Barents Sea and the donor populations. However, analysis of all the five loci for the pairwise differentiation between populations (Fisher's exact test) showed significant differences between some of the samples (Table 4a). The locus *Pca103* displayed the most pronounced differences: the test for interpopulation differentiation has demonstrated that the Varrangerfjord sample of red king crab is significantly distinct from both the other samples of the Barents Sea and the Far Eastern populations (Table 4b). On the other hand, no significant differences at this locus were detectable for the rest five samples. We suggest that the microsatellite locus *Pca103* can be a genetic marker for distinguishing between the Varrangerfjord population and the other populations.

DISCUSSION

We have studied the polymorphism of the mitochondrial gene *COI* and nuclear microsatellite loci of the red king crab from the four introduced Barents Sea populations and two donor Pacific populations. Until recently, no sufficient attention was paid to studying the population structure of this species. Several papers have reported the allozyme variation of red king crab from the Alaskan coast [10], Primorye (Peter the Great Bay) [9], and Western Kamchatka shelf [11]. All authors reported a low allozyme variation at the majority of polymorphic loci; the least mean heterozygosity (0.008) is characteristic of the Western Kamchatkan red king crab populations. Other Far Eastern crab species display a similar level of variation in allozyme markers [11].

Evidently, it was expedient to perform further population studies using DNA markers. For this purpose, species-specific primers for amplification of microsatellite loci were developed in 2002 [14], which made it possible to study the microsatellite polymorphism of three red king crab populations, namely, from the Alaskan coast, Western Kamchatka, and Varrangerfjord in the Barents Sea [12]. In this case, a special interest was not only in the interpopulation comparisons, but also in

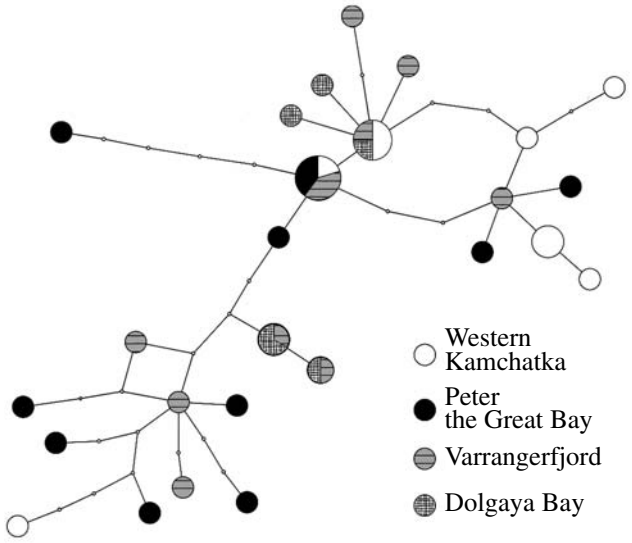

Fig. 3. Minimum spanning tree of mitochondrial haplotypes constructed based on a fragment of gene *COI*.

Table 4. Probability testing for pairwise differentiation between populations

(a) Based on five microsatellite loci

	V	Dl	T	D	K
Dl	0.00164**				
T	0.02647*	0.44584			
D	0.01070*	0.21498	0.60006		
K	0.00389**	0.09379	0.30630	0.02753*	
Pr	0.00017***	0.25327	0.00316**	0.00146**	0.38541

(b) Based on *Pca103* locus

	V	Dl	T	D	K
Dl	0.00135**				
T	0.00711**	0.67511			
D	0.01562*	0.42286	0.19610		
K	0.00073***	0.25197	0.55376	0.09417	
Pr	0.00050***	0.33149	0.54951	0.24586	0.61656

Note: Designations of samples are listed in Table 1.
*$P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$.

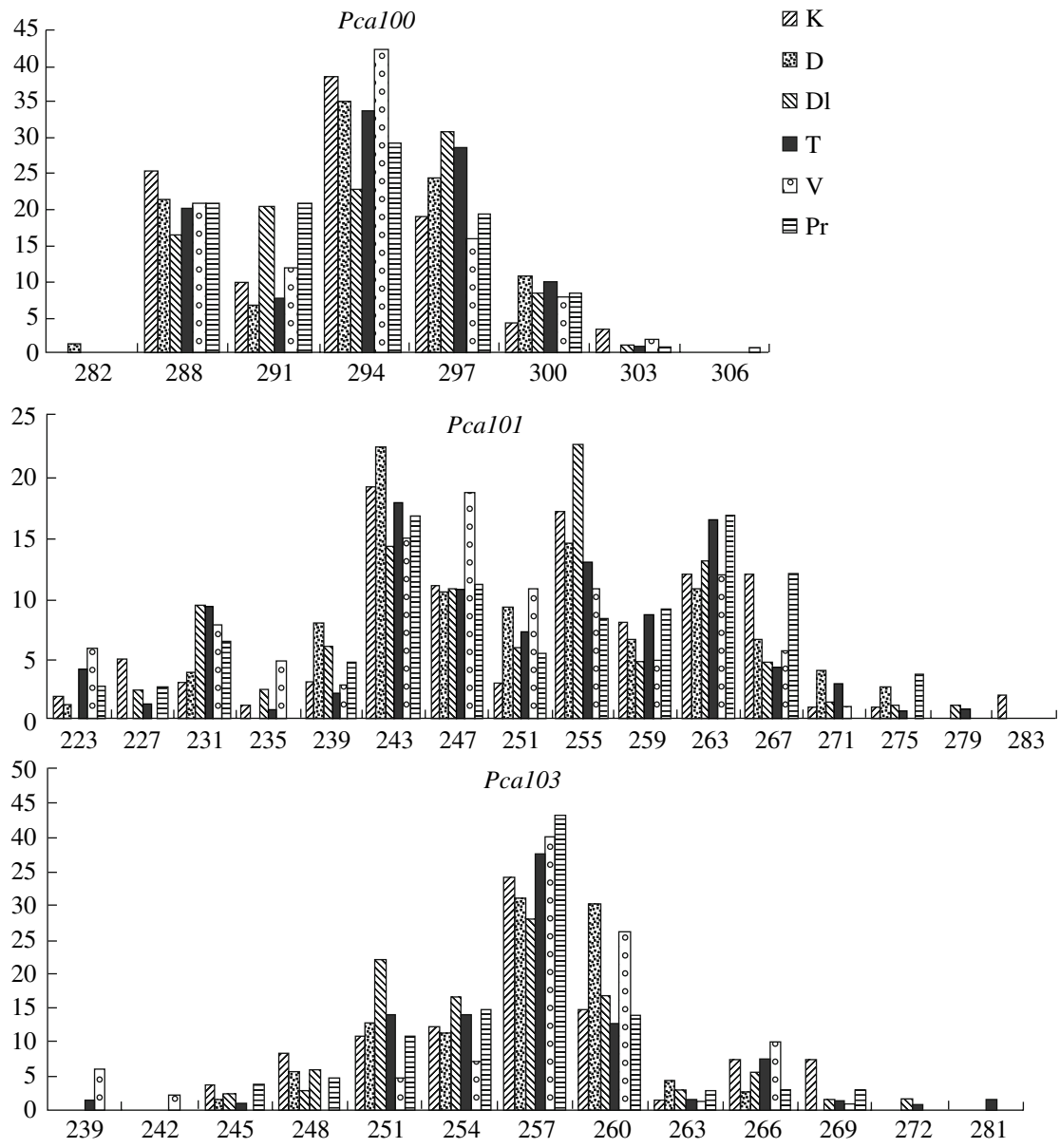

Fig. 4. Allelic frequency distributions for each analyzed locus in the samples from the Barents Sea (V, Varrangerfjord; Dl, Dolgaya Bay; T, Teriberka Bay; and D, Drozdovka Bay) and Far East (K, Western Kamchatka and Pr, Peter the Great Bay). The abscissa shows the lengths of alleles, hk; the ordinate, frequencies (%).

that the genetic polymorphism of "migrants" was analyzed along with the native populations.

Recently, the interest to the subject of successful introduction of red king crab to the Barents Sea has essentially increased; correspondingly, genetic studies in this field are becoming ever more relevant. Note that the genetic processes in invasive populations are insufficiently studied, and each case of introduction of a species into a new environment is essentially unique. Two scenarios are possible in the invasive populations, namely, a decrease in genetic polymorphism due to a bottleneck effect and, on the contrary, an admixture effect, when individuals from different populations are the founders of new population within its natural habitation area [18]. Nonetheless, a founder effect is usually expected in such studies and, as a consequence, a decrease in the genetic diversity. In our case, the introduced populations retained the allelic polymorphism at

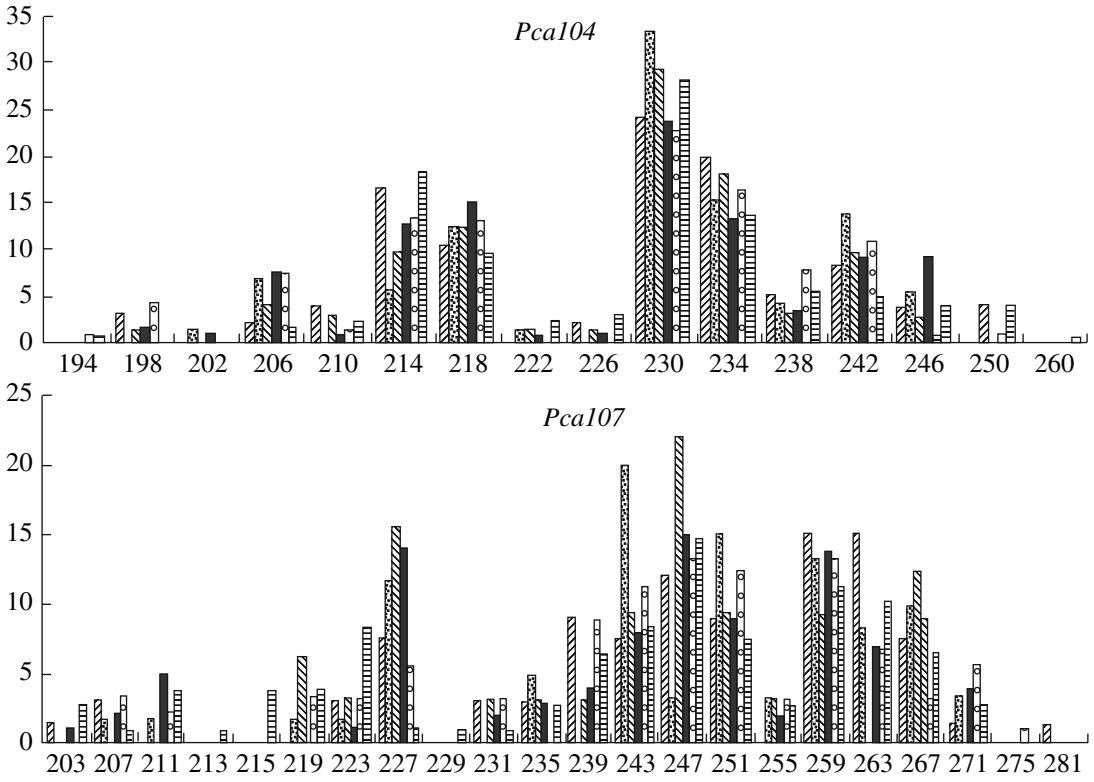

Fig. 4. Contd.

the level of donor populations; this is not surprising taking into account that the red king crab had been repeatedly introduced from the natural area during several years (1961–1969 and 1977–1978), and the genetic diversity of the introduced individuals was considerable. Two remote populations were used as donors during the acclimatization; thus, despite a generally accepted opinion, we could expect an increase in the genetic polymorphism in the introduced populations as compared with the donor populations. However, this effect can be observed only if the donor populations were genetically different, which was not the case for the Western Kamchatkan and Primorye populations.

Red king crab belongs to the species with a long generation time: the crabs reach sexual maturity at an age of 10–12 years. Thus, according to the hypothesis of the generation time effect, the rate of molecular evolution in this species must be lower than in the species with a short generation time [19]. This hypothesis can to a certain degree explain the obtained results on comparative analysis of polymorphism of two natural Far Eastern populations: despite that these populations are rather geographically remote, any significant genetic differences between them were undetectable in both *COI* and five highly polymorphic microsatellite loci. However, taking into account that the gene flow between individual red king crab populations is limited, as demonstrated by the studies of population structure [20] and the behavior of crab larvae [21], explanation of this phenomenon requires further studies.

The detected significant differences between some introduced populations and the native populations suggest that although the introduced populations retained their allelic diversity, gene drift is observed in some of their populations. The most pronounced differences (statistically significant) were found for the Varrangerfjord population. As Varrangerfjord is a rather hydrologically isolated bay, this suggest that a genetically isolated group could have developed there; thus, a gene drift and a certain founder effect is observable here without any decrease in the genetic polymorphism.

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