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ANIMAL GENETICS

Preliminary Data on Variation of Four Microsatellite Loci in Pacific Herring *Clupea pallasii*

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Abstract—Variation of microsatellite loci *Cpa110, Cpa113, Cpa4,* and *Cpa7* was for the first time examined in Pacific-type herring *Clupea pallasii* from the White Sea (*Cl. pallasii marisalbi*), the Kara Sea (*Cl. pallasii suworowi*), the Sea of Okhotsk, and Lake Nerpich'e, Kamchatka Bay, northwestern Pacific (*Cl. pallasii pallasii*). All loci exhibitedhigh genetic diversity. The estimates of expected heterozygosity varied from 41.5 to 95.6% (mean, 82%). The level of pairwise genetic differentiation F_{st} at all microsatellite loci varied from 0.005 to 0.076 (0.019, on average) and ~~t~~was statistically significant ($p < 0.05$) in most of the pairs of herring samples. Estimates of genetic differentiation among the herring of one subspecies were lower than between the groups belonging to different subspecies.

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INTRODUCTION

Pacific-type herrings (*Clupea pallasii*) inhabit the Arctic Ocean drainage from the White Sea to the east. They are abundant in the southeastern part of the Barents Sea, Chesha Bay, and Pechora Bay, while their number is lower in the southern regions of the Kara Sea. In the Pacific Ocean, these herrings are widely distributed along the American coast from California to Alaska, and along the Asian coast till the western coast of Korea. Three subspecies of *Clupea pallasii* are recognized, including the proper Pacific (*Cl. pallasii pallasii*), the Chesha-Pechora (*Cl. pallasii suworowi*), and the White Sea (*Cl. pallasii marisalbi*) herrings. Each of the subspecies within its range forms a great number of intraspecific groupings (schools, races), differing in the regions of habitation, morphometric characteristics, age structure, growth rate, age at sexual maturity, migration cycle features, spawning time, etc. In addition, in proper Pacific herrings (*Cl. pallasii pallasii*), two forms are distinguished, including sea herrings reproducing in the offshore zones and lake herrings, which live in sea, but enter brackish lakes to spawn [1]. However, along with clear biological differentiation, Pacific herrings demonstrate low levels of genetic divergence at different markers, like allozymes [2–6], mtDNA [7], ribosomal DNA [8], and microsatellites [9–11]. Despite of wide distribution of herrings, they are the subjects of few studies cited above. These studies mostly examine separate groupings of *Cl. pallasii pallasii* from the waters of Alaska, northern part of Bering Sea, and the offshore waters of Japan, providing no general pattern of the population genetic structure and relationships across the whole species range. Moreover, the herrings inhabiting the Arctic Ocean remain poorly investigated. In our earlier studies, population genetic structure of herrings from White Sea and southeastern part of Barents Sea was examined using allozyme markers [12–14]. In the present study, preliminary data on the microsatellite loci variation in the sea herrings from the White Sea, the Kara Sea, and the Sea Okhotsk are presented along with the similar data for the lake form of herring from Nerpich'e Lake, Kamchatka Bay, northwestern part of the Pacific Ocean.

MATERIALS AND METHODS

Polymorphism of microsatellite loci was studied in the herring samples from the White Sea, the Kara Sea, the Sea of Okhotsk, and from Nerpich'e Lake (northeastern part of Kamchatka Peninsula), northwestern part of the Pacific Ocean, Kamchatka Bay. The samples were collected in 2001 through 2010. In the White Sea, the samples were collected in the herring spawning season on the grounds located in the southern end of Onega Bay and in Kandalaksha Bay (Chupa Inlet). The samples were also taken from Kara Sea (near the settlement of Ust'-Kara) and the Sea of Okhotsk (Taui

Table 1. Characteristics of the material examined

Sampling locality	Sampling date, year	Coordinates		Systematic classification	Sample size
		latitude	longitude		
White Sea:					
Onega Bay	2006	63°56.8′N	37°58.47′E	*Clupea pallasii marisalbi*	50
Kandalaksha Bay (Chupa Inlet)	2001 2008	66°20.5′N	33°43.3′E	*Cl. pallasii marisalbi*	18 50
Kara Sea: Ust'-Kara	2008	69°17.3′N	65°3.2′E	*Cl. pallasii suworowi*	11
Sea of Okhotsk:					
Taui Bay	2009	59°42.5′N	149°20.5′E	*Cl. pallasii pallasii* (sea)	21
Northern part of the Pacific Ocean:					
Nerpich'e Lake (Kamchatka)	2010	56°21.2′N	162°38.3′E	*Cl. pallasii pallasii* (lake)	7

Bay). In addition, small sample of herrings from Nerpich'e Lake, Kamchatka Bay, northwestern part of the Pacific Ocean, was available for the analysis. A list of samples and their codes are presented in Table 1. Geographic localities of the samples are demonstrated in Fig. 1.

Tissue samples for the DNA analysis (pectoral fins, white skeletal muscles, or eyes) were fixed in 96% ethanol. Total DNA was extracted according to a standard method using the Diatom DNA Prep kit (IzoGen, Russia).

PCR amplification was performed using the GenePak PCR Core kits (IzoGen Laboratory, Russia). The reactions were run in a final volume of 20 μl of the incubation mixture contained PCR buffer, 200 μM of each deoxyribonucleotides (dTTP, dCTP, dATP, and dGTP), 1.5 mM $MgCl_2$, 50 ng genomic DNA, and 100 ng of a specific primer. Amplification of microsatellite loci was carried out using an MJ Research PTC-100 thermal cycler. The reaction conditions included denaturation for 2 min at 94°C, followed by eight cycles of denaturation of DNA template for 1 min at 94°C; primer annealing for 30 s at X°C; and extension for 30 s at 72°C. Then, followed 21 cycles of 30 s at 94°C; 30 s at X°C; 15 s at 72°C; and final extension at

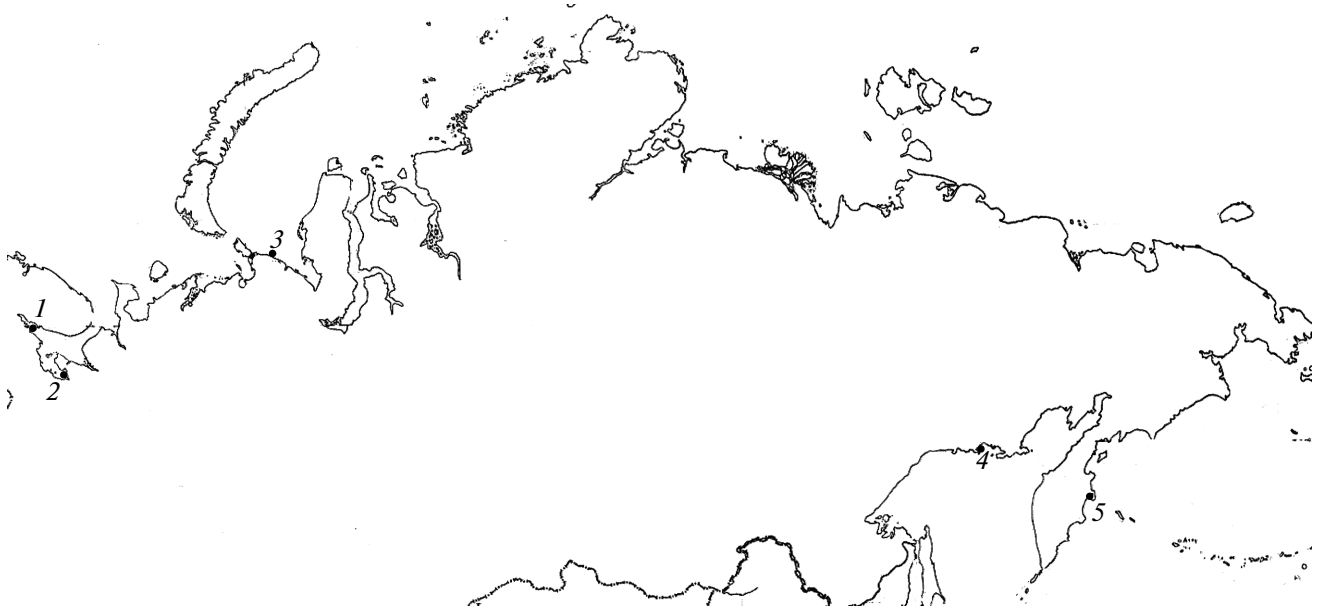

Fig. 1. Map showing geographic location of sampling sites: *1*, Kandalaksha Bay of the White Sea, Chupa Inlet; *2*, Onega Bay of the White Sea; *3*, Kara Sea, Ust'-Kara; *4*, Sea of Okhotsk, Taui Bay; *5*, Nerpich'e Lake, Kamchatka, Kamchatka Bay, northwestern part of the Pacific Ocean.

Table 2. Characteristics of microsatellite loci studied

Locus	Repeat unit	Primer sequence (5'–3')	Interval of allele sizes, bp	Literature source
Cpa110	$(TAGA)_n$	F: CTGACAACCCTCGACATACAT R: ACAATTTGCACTGGTTTGTAGTAG	138–162	[15]
Cpa113	$(ATCT)_n$	F: TGTCCATCTGTCCATTCAGC R: ACCACACAGCACATTTACAGG	120–204	[15]
Cpa4	$(GACA)_n$	F: CTTATCTGTCTGACTGCCTATTTG R: GTTTCTTCTCTGCTCCACCCAGAA	104–200	[16]
Cpa7	$(GATA)_n$	F: GGTATTGTGTTTGACAAACT R: GTTTGTAAGTGTATAAGCTACTA	110–244	[16]

72°C for 3 min. The annealing temperature for individual primer pair was 52°C.

Amplification products were separated by means of electrophoresis in 6% nondenaturating polyacrylamide gel in 0.5×TBE buffer at 300 V for 2 to 3 h. The gels were stained with ethidium bromide and photographed in UV light. DNA of pBr322 plasmid, digested with either *Hae*III or *Hpa*II restriction endonuclease, was used as the molecular size marker. Allele sizes at each locus were determined using the 1D Image Analysis Software Version 3.5 program (Kodak).

Herring variation was evaluated using four microsatellite loci, *Cpa110*, *Cpa113*, *Cpa4*, and *Cpa7*. The loci characteristics are presented in Table 2. From the literature data it is known that the *Cpa7* repeat unit is $(GATA)_n$. In other words, the size difference between the alleles should be multiple of four nucleotides. At the same time, at this locus, alleles differing in two nucleotides were identified. Because of this, allele sizes at this locus were determined with the interval of two nucleotides. An example of electrophoregram of the *Cpa7* amplification products is presented in Fig. 2.

Estimates of allele frequencies, allelic diversity, and expected and observed heterozygosity, as well as statistical tests for conformance of genotype distributions at each locus to Hardy–Weinberg equilibrium (intrapopulation inbreeding coefficients *f*) [17] were performed using the GDA software program [18]. The GENEPOP 3.4 package [19] was used to evaluate linkage disequilibrium, the degree of population differentiation F_{st}, and to calculate the Nei's genetic identity [20]. Statistical significance of pairwise genetic differentiation between the samples was evaluated using the χ^2 test. All probability tests were based on the Markov chains method [21]. Statistical significance of the F_{st} values was evaluated using the Arlequin 3.11 software package [22]. The degree of population differentiation

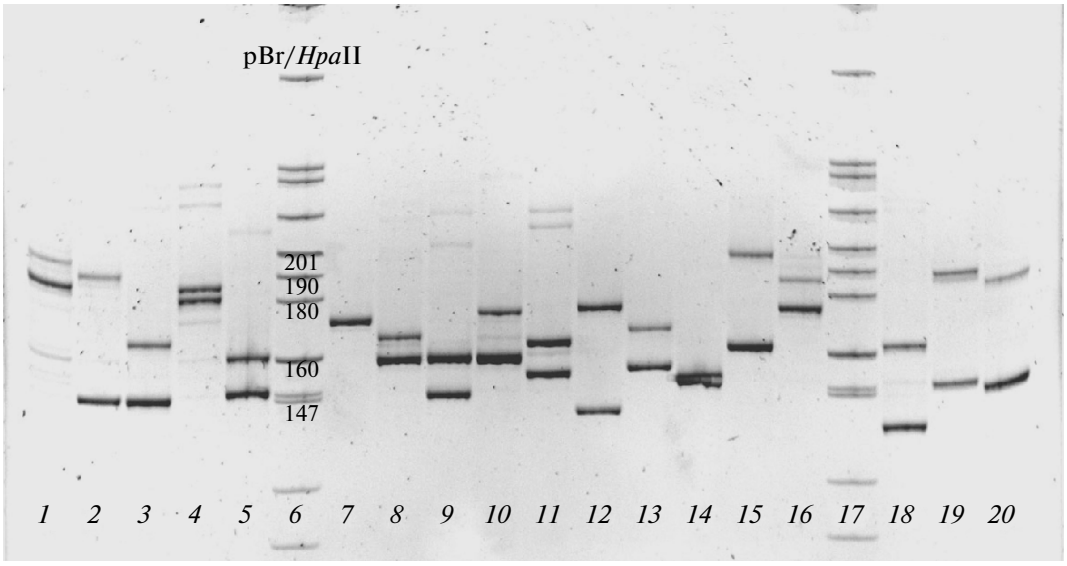

Fig. 2. Example of polymorphic distribution of STR patterns obtained with primer @Cpa7@. Lanes 6 and 17, molecular size marker, DNA of pBr322 plasmid digested with the @Hpa@II restriction endonuclease; Distribution of genotypes along the lanes: 1, 186/196; 2,146/190; 3, 146/166; 4, 180/186; 5, 150/162; 7, 174/174; 8, 160/168; 9, 150/160; 10, 160/176; 11, 154/164; 12, 144/178; 13, 156/168; 14, 152/154; 15, 162/198; 16, 176/186; 18, 140/162; 19, 150/188; 20, 148/184

Table 3. Herring population genetic variation of at microsatellite loci

Locus	Indices	Samples					
		Onega Bay	Chupa Inlet 2001	Chupa Inlet 2008	Kara Sea	Sea of Okhotsk	Nerpich'e Lake
Cpa110	*n*	50	18	50	11	21	7
	A	4	2	5	2	4	2
	H_E	0.579	0.500	0.498	0.415	0.558	0.439
	H_O	0.520	0.389	0.540	0.545	0.666	0.571
	f	0.102	0.227	–0.084	–0.333	–0.199	–0.333
	p	0.001*	0.368	0.519	0.528	0.382	1.000
Cpa113	*n*	50	18	48	11	21	7
	A	14	10	13	7	13	9
	H_E	0.909	0.857	0.883	0.736	0.909	0.945
	H_O	0.880	0.611	0.854	0.727	0.762	1.000
	f	0.032	0.293	0.033	0.012	0.165	–0.063
	p	0.054	0.001*	0.711	0.681	0.030*	0.404
Cpa4	*n*	50	18	50	11	21	7
	A	15	13	15	7	15	8
	H_E	0.907	0.876	0.872	0.731	0.928	0.901
	H_O	0.960	1.000	0.940	0.636	0.905	1.000
	f	–0.058	–0.146	–0.078	0.135	0.025	–0.120
	p	0.748	0.931	0.059	0.306	0.399	0.721
Cpa7	*n*	49	18	49	11	20	7
	A	25	19	24	14	22	10
	H_E	0.939	0.941	0.934	0.956	0.953	0.945
	H_O	0.979	0.944	0.898	1.000	1.000	1.000
	f	–0.043	–0.003	0.038	–0.047	–0.049	–0.063
	p	0.744	0.807	0.466	1.000	0.201	0.444

Notes: *A*, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; *f*, intrapopulation coefficient of inbreeding; *p*, probability of conformance to Hardy–Weinberg equilibrium.

* Statistically significant deviation from Hardy–Weinberg equilibrium.

was also evaluated using θ (analogue of F_{st} [17]) statistics as implemented in the GDA program.

RESULTS AND DISCUSSION

All microsatellite loci examined were polymorphic. The number of alleles per locus varied from six (*Cpa110*) to 35 (*Cpa7*) (20, on average). The total number of different alleles identified was 80. In each of the loci examined in herrings, specific alleles observed in a single sample were detected. Five alleles with low frequencies (from 0.01 to 0.04) were detected in each of the herring samples from Onega Bay (*Cpa113*, *Cpa7*), Chupa Inlet (*Cpa113*, *Cpa4*, and *Cpa7*), and the Sea of Okhotsk (*Cpa110*, *Cpa113*, *Cpa4*, and *Cpa7*). One specific allele at the *Cpa113* locus was detected in lake herrings from Kamchatka with the frequency far higher than that of the loci described above (0.143).

In the herring samples tested, the genotype distributions at nearly all loci were in Hardy–Weinberg equilibrium. Several exclusions were observed. Specifically, statistically significant deviation from the equilibrium ($p < 0.05$) in the genotype distribution was observed at the *Cpa110* locus in herrings from Onega Bay, and at the *Cpa113* locus in the sample from Chupa Inlet (2001) and the Sea of Okhotsk. In all these cases, the deficit of heterozygotes was revealed. The test for linkage disequilibrium revealed no correlation between the genotypes at any of the loci.

The value of expected heterozygosity in the samples ranged from 0.415 in *Cpa110* to 0.956 in *Cpa7* locus (Table 3). The value of mean expected heterozygosity was the lowest in the herrings from Kara Sea (H_E = 0.710), while it was the highest in the herrings from

Table 4. Nei's genetic identity values [20] (above the diagonal) and the F_{st}-based herring genetic differentiation values (below the diagonal)

Samples	Onega Bay	Chupa Inlet 2001	Chupa Inlet 2008	Kara Sea	Sea of Okhotsk	Nerpich'e Lake
Онежский залив	–	0.911	0.916	0.838	0.873	0.768
Губа Чупа, 2001 г.	0.005	–	0.903	0.781	0.820	0.737
Губа Чупа, 2008 г.	**0.011**	0.008	–	0.788	0.846	0.785
Карское море	**0.037**	**0.048**	**0.050**	–	0.712	0.652
Охотское море	**0.010**	**0.019**	**0.021**	**0.068**	–	0.733
Озеро Нерпичье	**0.022**	**0.027**	**0.022**	**0.076**	**0.025**	–

Note: The F_{st} values statistically significant at $p < 0.05$ are in bold type.

Taui Bay, the Sea of Okhotsk ($H_E = 0.837$), as well as in those from Onega Bay, White Sea ($H_E = 0.834$). In the herrings from Chupa inlet (2001 and 2008) and in lake herrings from Kamchatka the values of this index were very close and varied from 0.794 to 0.807.

Multiple testing for genetic differentiation (Fisher's exact test) using the *Cpa110* allele frequencies showed no statistically significant differences between all sample pairs examined. In the *Cpa113* locus, in most of the cases statistically significant ($p < 0.05$) differences in allele frequencies between the pairs of all localities were observed. Only the differences between the Chupa Inlet (2001) and Onega Bay samples, as well as between the Chupa Inlet (2001) and Sea of Okhotsk samples were not statistically significant. In *Cpa4* and *Cpa7* loci, the differences were statistically significant in 50% of all comparisons. However, comparisons over all four microsatellite loci showed the absence of statistically significant differences only between the herring from Onega Bay and the samples from Chupa Inlet and Kara Sea.

The degree of genetic differentiation among all herring samples examined in the θ values was statistically significant and constituted 1.84% with 95% confidence bootstrap interval [0.43; 3.31]. The microsatellite loci examined were found to be different in the levels of genetic differentiation. The highest contribution to the differentiation between the samples was made by the *Cpa113* locus: θ = 3.75%. Estimates for the *Cpa110* locus were the lowest: θ = 0.27%. The θ values for the *Cpa4* and *Cpa7* loci constituted 1.99 and 0.72%, respectively. In the White Sea (*Cl. pallasii marisalbi*) estimates of heterogeneity between the herring samples were not statistically significant, θ = 0.87% [–0.07; 2.15]. The degree of differentiation between sea herrings from the Sea of Okhotsk and lake herrings from Kamchatka within the limits of the subspecies *Cl. pallasii pallasii* was higher, θ = 2.35% [1.54; 3.45] and statistically significant.

Testing of the herrings from Kamchatka, the Sea of Okhotsk, and the Kara Sea for heterogeneity yielded the θ value of 5.27% [0.93; 7.79].

Nei's genetic identity values [20] between the herring groupings were calculated based on four microsatellite loci data. The index values varied from 0.652 to 0.916. The highest identity values were observed between the White Sea herring, from 0.903 to 0.916. Lake herring from Kamchatka demonstrated the lowest level of similarity to the other groupings tested (0.652 to 0.785).

The level of pairwise differentiation between the samples in F_{st} values across all microsatellite loci varied from 0.005 to 0.076, with the average value of 0.019 (Table 4). Differentiation between the samples was statistically significant in most of the comparison pairs, excluding the differences between the Chupa Inlet herring samples of 2001 and 2008, and the Chupa Inlet herring sample of 2001 and the sample from Onega Bay.

Thus, the Pacific-type herring groupings examined demonstrated the high level of genetic diversity at four microsatellite loci. Estimates of the mean expected heterozygosity varied from 0.710 to 0.837, with the average value of 0.820. The data obtained are comparable with the genetic diversity estimates inferred from microsatellite loci data for the Pacific herrings from the shoreline of Alaska (0.893 to 0.903) [9], California and British Columbia (0.840 to 0.890) [23], Japan (0.782 to 0.945) [11], and for the Atlantic herrings *Clupea harengus*: 0 (0.90 to 0.93) [24]. The mean heterozygosity values obtained in the present study were somewhat lower than those reported in the studies cited, because less polymorphic *Cpa110* locus was included into investigation. After the exclusion of the *Cpa110* locus, estimates of mean expected heterozygosity turned to considerably higher value of 0.918.

The herring from Kara Sea, compared to other groupings examined, demonstrated lower estimates of genetic diversity at the *Cpa110*, *Cpa113*, and *Cpa4*

locus, while the value of this index at the *Cpa7* locus was higher than in the other groups. Our earlier studies demonstrated lower polymorphism level at the allozyme loci in *Clupea pallasii suworowi* herrings from the Chesha–Pechora region [14] and Kara Sea (unpublished data), compared to the White Sea herring.

The pairwise F_{st} values visualizing genetic differentiation between all herring groupings examined varied from 0.005 to 0.076 (mean, 0.019) and were statistically significant ($p < 0.05$) in most of the comparison pairs, pointing to genetic differences between the herring groupings. The literature data on genetic differentiation in the F_{st} values in the Pacific herring were similar to our estimates. For instance, the differentiation index value for the herring from the coast of Alaska, California, and British Columbia constituted 0.003, on average, while for the herring of British Columbia it was 0.023 [23], and for the herring from the north of Japan, 0–0.044 [11].

Thus, preliminary data on the microsatellite loci variation showed their applicability for the analysis of the population genetic structure of Pacific herring and for identification of heterogeneity across the range. It was demonstrated that the degree of identity between the White Sea herring was higher than between the herring groups from different regions. These findings point to lower genetic differentiation in the herring of one subspecies, compared to the groupings belonging to different subspecies. More precise evaluation of the population genetic structure of Pacific herring requires additional data on larger samples with the involvement of the greater number of microsatellite loci.

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