Russian population genetics study of jack mackerel in the South Pacific

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Purpose: to study the genetic polymorphism of the South Pacific jack mackerel.

Material and methods. The sampling sites are shown in Table 1 and Fig. 1. The samples were collected in the Southeast Pacific from the Russian R/V "Atlantida". The samples in the Southwest Pacific were collected from fishing trawler "Professor M. Aleksandrov" of New Zealand on September 28, 2009 from 42°41' S., 170°05' E., and were kindly made available by Dr. A. Penney to the Russian side for analysis.

Table 1.

Jack mackerel sampling sites and number of samples taken for genetic analysis

	Latitude	Longitude	Date	Number of samples
Sample 1 Western	42.41 S	170.05 E	28.09.2009	80
Sample 2 Eastern	35.40 S	110.51 W	07.09.2009	60
	35.12 S	110.48 W	09.09.2009	20

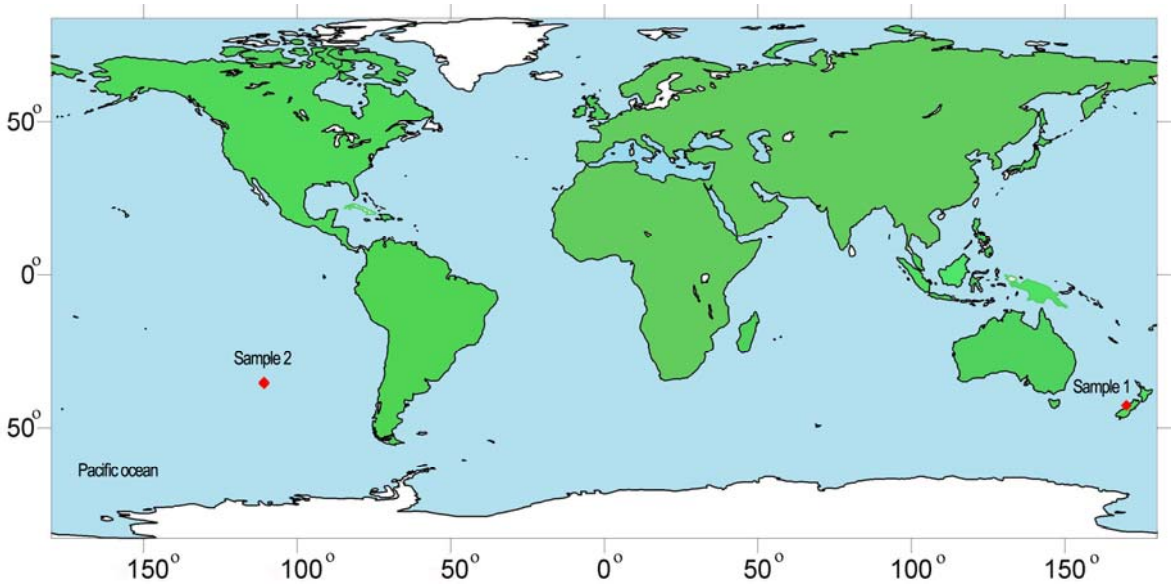

Fig. 1. Sampling map.

Molecular markers. For DNA analysis, tissue samples (mainly, a piece of the pectoral fin) were fixed in 96% ethanol. Total DNA was extracted with a standard isolation procedure with the Diatom DNA Prep reagent kit (IzoGen, Russia). PCR amplification was performed using the Gene Pak PCR Core reagent kit (IzoGen, Russia), with addition of 5 μl of primer mixture (final concentration 0.5 μM) and 5 μl of DNA template (100 ng). Microsatellite loci were amplified in a Veriti 96 thermal cycler (Table 2). Amplification products were fractionated by electrophoresis in the 6% nondenaturing polyacrylamide gel in 1xTBE buffer at 300V for 2 to 3 h. The gels were stained with ethidium bromide and photographed in the UV light. The 25-bp and 100-bp molecular weight standards (Promega, the United States) and pBr322 plasmid DNA digested with the *Hae* III and *Hpa* II restriction endonuclease were used as molecular weight markers. Allele sizes for each locus were determined using the 1D Image 6 Analysis Software, version 3.5 (Kodak).

All individuals were typed with microsatellites *TmurB2, TmurB104, TmurB116, TmurC4* (Canales-Aguirre et al., 2009). Primers for *TmurC4* were modificate in laboratory IzoGen, Russia. The loci and primer sequences are listed in Table 3. In this work we used one previous marker (*TmurC4*) and three new.

Table 2.
Programme for setting probes in amplification of microsatellite loci of the Pacific jack mackerel

Step	T, °C	Duration	Parameters
1	95	2'	
2	95	30"	
3	58	30"	
4	72	30"	
5	repeated 2-4		35 cycles

Table 3.
List of microsatellite loci and sequences of primers for their amplification

Name of locus	Sequences of primers	Sourse
TmurB104	F: TGAAGCACAAGTTTCCAAATC R:AAAGGTCAGAGAGAGAACAACG	Canales-Aguirre et al., 2009
TmurB116	F: CTCTCGTCTTCATTGAGGTCAC R:AAGTCGTCTGACTCATCTGTGC	Canales-Aguirre et al., 2009
TmurB2	F: AATCTTCATGTCACATAAACAC R:TCGACACTGTTGAGTCATC	Canales-Aguirre et al., 2009
TmurC4	F:CTCCTCTCACATTGCCCAT R:GTTCTTTCCAGCACTAATGGAA	Canales-Aguirre et al., 2009

The number of alleles, the expected (He) and observed (Ho) levels of heterozygosity, deviations from Hardy-Weinberg equilibrium were obtained using GDA (Lewis P.O, Zaykin D. 2001). Significance levels of populations differences (by Fisher's criterion) estimated in METROP (Guo, Thompson, 1992; Zaykin et al., 1995).

All loci were polymorphic. Basic genetic characteristics present in Table 4.

Table 4.

Characteristics of the microsatellite loci isolated for the Pacific jack mackerel

Locus	Repeat unit	Size range	No of alleles	N	He	Ho	F	H & W
Sample 1-Western								
TmurB2	$TG_{(5)}$ $AG_{(2)}TG_{(5)}$	138-150	6	47	0,602	0,319	0,473	0,000
TmurB104	$ATC_{(14)}$	144-171	10	68	0,635	0,412	0,354	0,000
TmurB116	$ATC_{(7)}$	144-153	5	64	0,404	0,422	-0,043	0,804
TmurC4	$CATC_{(9)}$	74-126	14	73	0,830	0,781	0,059	0,296
Sample 2 - Eastern								
TmurB2	$TG_{(5)}$ $AG_{(2)}TG_{(5)}$	138-150	8	67	0,723	0,448	0,382	0,000
TmurB104	$ATC_{(14)}$	144-171	10	65	0,766	0,600	0,218	0,000
TmurB116	$ATC_{(7)}$	144-153	5	71	0,381	0,394	-0,034	0,680
TmurC4	$CATC_{(9)}$	74-126	11	77	0,816	0,701	0,142	0,003

The number of alleles per locus ranged from 5 to 14, corresponding to markers *TmurB116* and *TmurC4*, respectively, and observed heterozygosity values from 0,319 to 0,781, for *TmurB2* and *TmurC4*, respectively. *TmurB2* and *TmurB104* showed significant deviation from Hardy-Weinberg equilibrium ($P<0,05$) in both samples. The null alleles or specimen from the different populations at the same sample may be the cause of it. *TmurC4* showed also significant deviation from Hardy-Weinberg equilibrium in sample 2. We found significant differences between localities. The value of Qp for *TmurB104* was 1,73% and the value of for *TmurB2* Qp was 2,17%. The average value of Qp for these two loci was =1,95%. We made four pairwise tests. Two tests showed significant differences between two samples according allels frequencies *TmurB104* and *TmurB2* at the 5% nominal level. Combine test for all loci showed significant differences between localities (p=0,0068).

On Figure 2-5 present loci electrophoregrams.

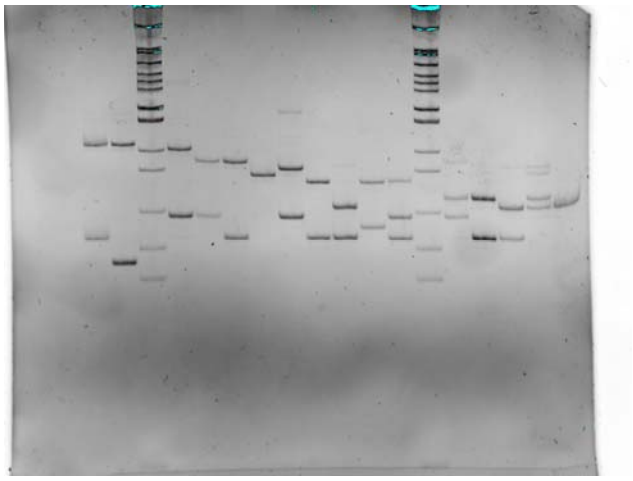

Fig. 2. Electrophoregram of *TmurC4.*

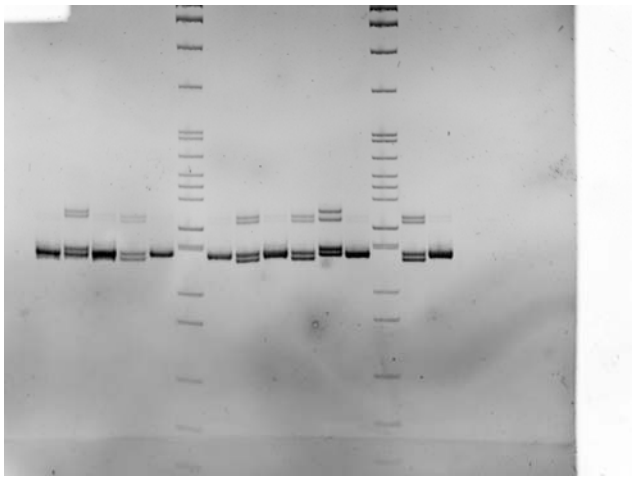

Fig. 3. Electrophoregram of *TmurB116*

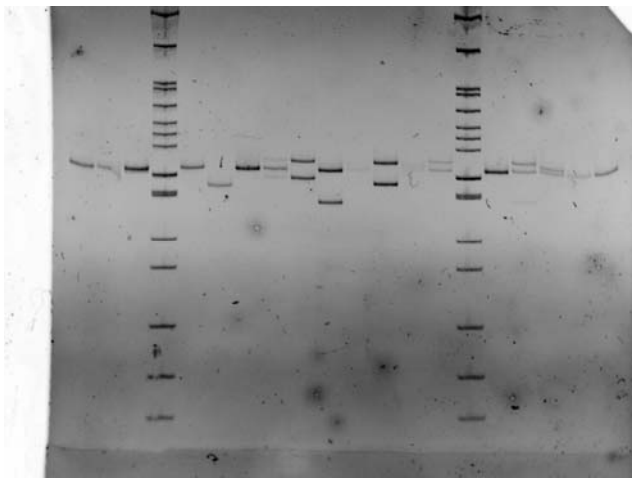

Fig. 4. Electrophoregram of *TmurB104*

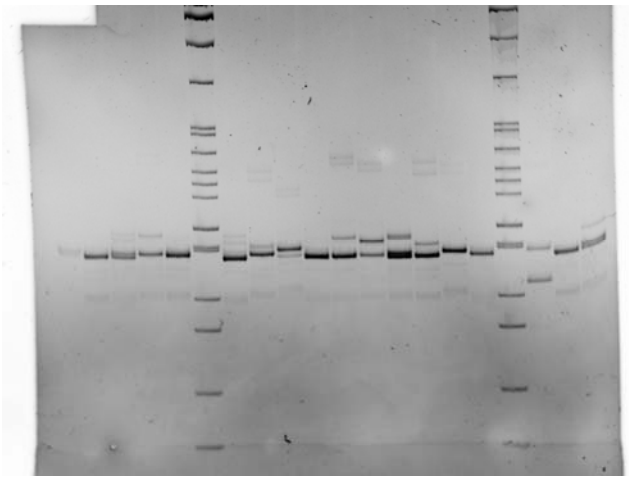

Fig. 5. Electrophoregram of *TmurB2*

In future we are going to study more microsatellite loci such as *Tt29, Tt74, TmurB6, KTj5* and third sample of jack mackerel.