

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. 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 41°46’ S., 170°48’ 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
41.46 S	170.48 E	28.09.2009	45
35.04 S	94.30 W	07.09.2009	20
35.12 S	92.59 W	09.09.2009	21
35.16 S	92.0 W	11.09.2009	12

The genetic study of jack mackerel *Trachurus morphyi* included microsatellite analysis and evaluation of amplified fragment length polymorphism (AFLP).

Microsatellite analysis. The microsatellite analysis became the “gold standard” in population research. The great polymorphism of microsatellite loci makes it possible to apply this technique to differentiating close populations or even subpopulations with a high level of confidence. The genetic distances are computed according to the differences in frequency of occurrence within the microsatellite allele samples.

One factor restricting the microsatellite technique is the difficulty to find species-specific microsatellite loci, so using loci references to analyze one’s own data is a common practice. Twelve microsatellite loci deposited in the nucleotide sequence genebank (<http://www.ncbi.nlm.nih.gov/genbank/>) are known for the Pacific jack mackerel. Eight loci were selected for analysis, as based on the testing results of primer sequences, to amplify each of them with the aid of targeted software. The loci and primer sequences are listed in Table 2.

Table 2.

List of microsatellite loci and sequences of primers for their amplification. Shown in colour are sequences of nucleotides homologous to fluorescently marked primer used to visualize PCR products in capillary electrophoresis

Name of locus	Sequences of primers	Source
TmurC4	TmurC4_FG CAGGAAACAGCTATGACccagagcgtactgtctggctg TmurC4_R tccagcactaatggatggttg	Canales-Aguirre et al. 2010
TmurA104	TmurA104_FG CAGGAAACAGCTATGACtcaccccaaattgtttgcca TmurA104_R ttactgtcaccaaagaccgca	Canales-Aguirre et al. 2010
TmurA115	TmurA115_FB GTTTCCCAGTCACGACtcaatagacgcagacatctgg TmurA115_R cttcgattacaaaggcagtgc	Canales-Aguirre et al. 2010

TmurB116	TmurB116_FB GTTTTCCCAGTCACGAC ggcattcatgactctcgtcttc TmurB116_R_cagctcggatgtttcaacagt	Canales-Aguirre et al. 2010
Tt29	Tt29_FY GTAAAACGACGGCCAGT actgacgaaggaacatgc Tt29_R_gtgcaggtaagccagctac	Cardenas et al. Unpublished
Tt48	Tt48_FY GTAAAACGACGGCCAGT ctcctcatatcccctctgttg Tt48_R_attcgatgtgcaaacaccac	Cardenas et al. Unpublished
Tt62	Tt62_FG CAGGAAACAGCTATGAC gtcagggtgctctgggtgc Tt62_R_ctctggcgtgacaggaatg	Cardenas et al. Unpublished
Tt133	Tt133_FB GTTTTCCCAGTCACGAC gatctcagactctccaccgta Tt133_R_agcacatccacacacttcca	Cardenas et al. Unpublished

Three loci (TmurC4, Tt29 and Tt133) were selected from the above by way of a trial PCR on a small sample (32 specimens). This makes it possible to obtain the most reproducible results which may be interpreted. The primers for microsatellite analysis were modified in a special way. The essence of this modification is that one of the primers (straight) in each pair is supplemented on the 5' carbon atom end with a universal sequence 17 nucleotide long (see Table 1). Three types of such sequences are used. At the same time a respective oligonucleotide with a fluorescent mark on 5' carbon atom end is added to the reaction. This approach enables us to obtain an unlimited number of various fluorescently marked PCR products having synthesized only three marked oligonucleotides.

PCR probes contained 50-150 ng DNA and were done in the volume of 15 µl (70 mM Tris HCL pH 8.3), 16.6 mM (NH₄)₂ SO₄, 1.8 mM MgCl₂, 200 µM of each dnpts, 0.5 µM of fluorescent primer, 1 µM of forward, and 1.5 µM of reverse primers, 0.8 Taq-polymerase), 0.2 µl of pyrrolydone. The probes were made in a Peltier thermocycle RTC 225 MJ Research according to programme given in Table 3.

Table 3.

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

Step	T, °C	Duration	Parameters
1	95	3'	
2	90	20"	
3	65	1'	-0.3 °C /step
4	72	30"	
5	repeated 2-4		20 cycles
6	90	20"	
7	59	1'	
8	72	30"	
9	repeated 6-8		10 cycles
10	90	20"	
11	59	1'	-0.2 °C /step
12	72	30"	

13	repeated 10-12		15 cycles
14	90	20"	
15	56	1"	
16	72	30"	
17	repeated 14-16		10 cycles
18	72	5'	
19	10	1'	
20	END		

The loci were amplified jointly. PCR products were reprecipitated for electrophoretic analysis; they were diluted in water and fractioned using capillary electrophoresis with ABI 3100 (Applied Biosystems). The electrokinetic injection of the examined DNA was preceded by adding HiDi formamide (10 mkl per 3 mkl of PCR product) and denatured at 96°C for 2 minutes. A marker modified with a fluorescent colourant ROX was used for inner DNA fragment length standard. The lengths of alleles were found using Gene Marker Software (SoftGenetics).

GENEPOP 3.4 (Raymond and Rousset, 1995) and Structure software (Pritchard, 2000) packages were applied to make statistical calculations of the microsatellite analysis data.

AFLP. It is obviously desirable to use different markers in order to trace differences between close groups. In the case of genetic studies of the Pacific jack mackerel the microsatellite analysis was complemented with the genome fingerprint technique in one of its most "authoritative" versions: AFLP. Amplification fragment length polymorphism (AFLP) is the most reproducible method of "full genome analysis", and is commonly used in population genetics of plants, though it is quite applicable to population studies of animals. The most significant advantage of this method in our case it that there is no need for a priori knowledge of the object's genome, i.e. we do not have to know the nucleotide sequence of particular loci, unlike the microsatellite markers. The method allows us to analyze a large number of nucleotide polymorphisms without knowing the extent to which the object has been studied; this is a considerable advantage since the genome of Pacific jack mackerel has not been sufficiently investigated while the absence of the necessary quantity of published molecular markers may be an obstacle to a profound study of the item's population structure.

The essence of the method is that anonymous parts of genome are selectively amplified using a series of consecutive stages; the closer the relations among the specimen examined are the more coinciding fragments can be recorded on electrophoregram after separation of the products of amplification.

Procedurally the technique includes two stages.

At first, the genome DNA was processed with restriction enzymes EcoRI and MspI which recognize the six-and-four nucleotide sequences. Adaptors were ligated to the fragments obtained.

Next, two successive amplification cycles were run where we used the primers complementary to the adaptor sequence which go beyond the restriction site by three nucleotides chosen arbitrarily. The triplets ensure amplification of some part of the restriction fragments only.

Sequences of the oligonucleotides used are given in Table 4.

Table 4.

Characteristics of the probes made during AFLP analysis of oligonucleotides

Name	Sequence of primer	Purpose of primer
AdEco-F	5'-CTC GTA GAC TGC GTA CC-3'	Forward adaptor EcoRI oligonucleotide

AdEco-R	5'-AAT TGG TAC GCA GTC TAC-3'	Reverse adaptor EcoRI oligonucleotide
AdMsp-F	5'-GAC GAT GAG TCC TGA G-3'	Forward adaptor MspI oligonucleotide
AdMsp-R	5'-TAC TCA GGA CTC AT-3'	Reverse adaptor MspI oligonucleotide
PrEco-A	5'-CTC GTA GAC TGC GTA CCA-3'	Preselective EcoRI primer
PrMsp-A	5'-GAC GAT GAG TCC TGA GA-3'	Preselective MpsI primer
PrEco-AAG	5'-CTC GTA GAC TGC GTA CCA Ag-3'	Selective EcoRI primer
PrMsp-ACC	5'-GAC GAT GAG TCC TGA GAC C-3'	Selective MpsI primer

An enzyme mixture is made to prepare for one restriction ligase probe:

- T4 buffer 10X 0.1 mcl
- Nacl 0.5M 0.1 mcl
- BSA 1mg/ml 0.05 mcl
- Msel: 1U 0.1 mcl
- EcoRI: 5U 0.12 mcl
- T4 ligase: 1U 0.2 mcl
- Water 0.33 mcl
- Total for one probe 1 mcl

The use of this aliquot leads to a probe of restriction-ligation:

- T4 buffer 10X 1 mcl
- Nacl 0.5M 1 mcl
- BSA 1mg/ml 0.5 mcl
- Enzyme mix 1 mcl
- MpsI adaptor 1 mcl
- EcoRI adaptor 1 mcl
- Total: 5.5 mcl
- DNA 5.5 mcl (concentration about 10 ng/mcl)

Composition of the final amplification probe is given in Table 5

Table 5.

Probe mix ingredients for AFLP analysis

Reagent	Stock concentration	Final concentration	Volume (mcl)
Taq buffer	10X	1X	1.25
Polymerase	5 u/mcl	0.25u	0.1
MgCl ₂	50 mM (check)	2 mM	0.5
dNTP	5mM	0.12 mM	0.3
primer-Msp	10µM	0.2 mM	0.25
primer EcoR	10µM	0.04 mM	0.05
BSA	1 mg/ml	8 mg/mcl	0.1
1/5 previous cycle probe			2.5

H ₂ O			Total = 12.5
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The terms of probe are given in Table 6

Table 6.

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

Pitch	T, °C	Duration	Parameters
1	94	2'	
2	94	30"	
3	65	30"	-0.7 °C /pitch
4	72	2"	
5	repeat pitch 2-4		12 cycles
6	94	30"	
7	56	30"	
8	72	2"	
9	repeat pitch 6-8		23 cycles
10	72	10'	

The electrophoretic analysis of amplification products was made with ABI 3100 (Applied Biosystems), as is described for microsatellite analysis.

Data were processed with Structure software (Pritchard, 2000).

At present it is envisaged to engage in analysis the five known microsatellite loci of jack mackerel which remain unexamined (TmurA104, TmurB116, Tt48, Tt62). The results will be summarized and presented upon completion of the analysis of all the eight loci.