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Russian population genetics studies of jack mackerel in the South Pacific

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P.K.Afanasiev

M.A.Rabchun

A.I.Glubokov

Introduction. In 2008, in order to research the population structure of the South Pacific jack mackerel, VNIRO began studies of the genetic polymorphism of the South Pacific jack mackerel with the participation of scientists from the Belozersky Institute of Physico-Chemical Biology of MSU (first phase, 2008), then in collaboration with scientists from the Russian laboratory "Genetic problems of identification", VIGG RAS (since 2010). The purpose of the research was to study the genetic polymorphism of microsatellite loci of the South Pacific jack mackerel.

Following loci were selected during the second phase of the study: TmurB2, TmurB104, TmurB116, TmurC4 (Canales-Aguirre et al., 2009). All loci were polymorphic. With a total of four loci analysis of differentiation is not reliable. Significant differences between localities ("Metrop" software) were estimated for two of the four loci. 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) (Buryakova, Afanasiev, Glubokov, 2011).

Studies conducted during the period of end 2011 – autumn 2012 continue the study of the population structure, which was began in 2008. The aim of this research was to expand the set of microsatellite markers for the purpose of a more complete analysis of the genetic population structure of the South Pacific jack mackerel.

Material and methods. The samples were collected in the Southeast Pacific aboard the Russian R/V "Atlantida", Russian F/V "Leader" and in the Southwest Pacific aboard New Zealand F/V "Professor M. Aleksandrov" (Fig. 1, Table 1). Southwest Pacific samples were kindly made available to the Russian side for analysis by Dr. A. Penney.

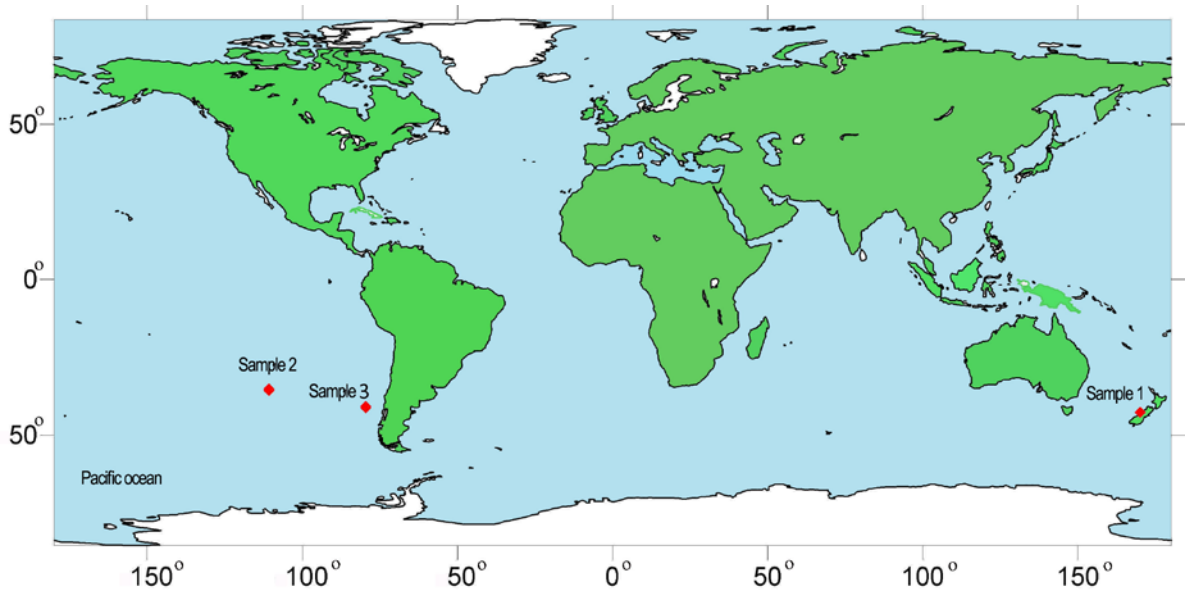

Fig. 1. Sampling map

Table 1. Characteristics of Jack mackerel genetic sampling

	Vessel name	Flag State	Latitude	Longitude	Date
Sample 1 Southwestern Pacific	Professor M. Aleksandrov	New Zealand	42.41 S	170.05 E	28 September 2009
Sample 2 Southeastern Pacific	Atlantida	Russian Federation	35.40 S	110.51 W	7 September 2009
	Atlantida	Russian Federation	35.12 S	110.48 W	9 September 2009
Sample 3 Southeastern Pacific	Leader	Russian Federation	38.13 S	83.44 W	March – May 2011

In 2011-2012 22 loci were chosen and tested. The loci and primer sequences are listed in Table 2.

In the first stage, two the most suitable for the further work tetranucleotide loci of the jack mackerel additional for the previous studies were selected in the NCBI database (Tt48, Tmur C12). In order to reduce the size of the PCR product and increase the reliability of amplification new forward and reverse primers for these loci were designed. The general pattern for the PCR cycle used for amplification of these microsatellite loci described in Programme №1 (Table 3).

The next step was to test 20 pairs of primers designed for relatively conservative loci previously used in the laboratory for the intraspecies structure studies of more than one species or sometimes more than one genus or even one order of fish (Table 4). The general pattern for the PCR cycle used for amplification of these microsatellite loci is described in Programme №2 (Table 3).

Table 2. List of microsatellite loci and sequences of primers for their amplification

№	Name of locus	Species	PCR programme	Annealing temperature	Sequences of primers	Source
1	Tt48 F	Trachurus trachurus	1	50°C 52°C 53°C 56°C 58°C 60°C	F: GCA-TCA-GAT-GTC-TCA-TAG-TGG-ATT R: CAA-ACA-CCA-CAT-GCA-TAC-ATA-CA	Kasapidis,P. and Magoulas,A., 2008
2	Tmur C12 F	Trachurus trachurus	1	50°C 52°C 53°C 56°C 58°C 60°C	F: TTC-TGG-TTC-TGT-TTG-AGT-CAT-AGA R: CCT-ATG-AGT-TAT-GTC-TAC-GCA-TAG-T	Canales-Aguirre,C.B., Ferrada,S. and Galleguillos,R., 2010
3	Omy 1011	Oncorhynchus mykiss	1 2	50°C 50 °C 52°C 54°C	F: AAC-TTG-CTA-TGT-GAA-TGT-GC R: GAC-AAA-AGT-GAC-TGG-TTG-GT	Gharrett et al. 2007
4	Ssa 419	Salmo salar	1 2	50°C 50°C 52°C 54°C	F: GGT-CGT-ATC-GCG-TTT-CAG-GA R: TGC-TGC-AAT-AAA-GAG-ATG-CTT-GTT	Gharrett et al. 2007
5	Oki 10*	Oncorhynchus kisutch	1	52°C	F: GGA-GTG-CTG-GAC-AGA-TTG-G R: CAG-CTT-TTT-ACA-AAT-CCT-CCT-G	Smith et al. 1998
6	Oki 23*	Oncorhynchus kisutch	1	52°C	F: CAT-CAC-ACG-CTT-CCT-AGA-GTG-A R: CCT-CAT-CCA-CGT-TAG-CAT-CA	Gharrett et al. 2007
7	OMM 1070	Oncorhynchus	1	50°C	F: GTT-GTG-TCG-AAT-	Rexroad III et

		mykiss	2	48°C 50°C 52°C 54°C	GGA-GTT-CTG R:GTG-CAC-TCT-AAA-TGA-ATC-AAT-CTC-TT	al. 2001
8	Ots 102*	Oncorhynchus tshawytscha	1	52°C	F: GGA-TCC-AAT-AAG-GAG-TGA-TAT-AGT-AG R: TAT-CCC-CTT-TAC-CAT-TTC-CCT-TGC-TA	Nelson et al. 1999
9	OMM 1037*	Oncorhynchus mykiss	1 2	50°C 50°C 52°C 54°C	F: GAA-CGG-CGA-CTG-GAT-TTA-ATA-CT R: CCG-CTC-ACC-CTC-GTC-TCT-TAA	Rexroad III et al. 2002
10	Oki1	Oncorhynchus kisutch	1	52°C	F: AGG-ATG-GCA-GAG-CAC-CAC-T R : CAC-CAT-AAT-CAC-ATA-TTC-AGA	Smith C.T., Koop B.F., Nelson R.J. 1998
11	One 104 G	Oncorhynchus nerka	1	52°C	F: GCT-ACT-ACA-ATC-CTA-GTC-TGT-GAT-T R: CAT-CTT-CTT-CAG-TGG-CTG-TAG-AT	Афанасьев П.К., Рубцова Г.А. и др., 2011
12	Gmo8	Gadus morhua	2	48°C 50°C 54°C	F: GCA-AAA-CGA-GAT-GCA-CAG-ACA-CC R: TGG-GGG-AGG-CAT-CTG-TCA-TTC-A	Miller,K.M., Le,K.D. and Beacham,T.D., 2000
13	Gmo19	Gadus morhua	2	48°C 50°C 52°C 54°C	F: CAC-AGT-GAA-GTG-AAC-CCA-CTG R: GTC-TTG-CCT-GTA-AGT-CAG-CTT-G	Miller,K.M., Le,K.D. and Beacham,T.D., 2000
14	PGmo32	Gadus morhua	2	50°C	F: CAA-TCG-CCG-TCC-AAC-CAA-C R: GGC-GGC-AGC-AAC-GAT-TCT-C	Jakobsdottir,K., Jorundsdottir,T. D., Skirnisdottir,S. et al, 2006
15	Gmo G18	Gadus morhua	2	48°C 50°C 52°C 54°C	F: ACA-GCA-GGT-AAT-GAG-ACA-AGC-AGA R: CTT-AGT-CTC-GTT-TGA-CCA-CAC-CTG	Wesmajervi M.S., Tafese T., Stenvik J. et al. 2007
16	Gmo G12	Gadus morhua	2	50°C	F: ATA-ACA-CTA-TTG-CAT-TCC-CTG-TGT R: TCA-GCG-GAA-TTA-GCA-ACT-TAA-AGA	Wesmajervi M.S., Tafese T., Stenvik J. et al. 2007
17	Afim 392	Anoplopoma fimbria	1	52°C	F: CAT-AGG-AAT-ACT-CCA-TTC-ATC-GT R: GGA-GCC-TCC-ATT-GAT-TAC-TTT	Messmer,A., Rondeau,E., Sanderson,D., Cooper,G.,

						Leong,J., Campbell,B., Koop,B.F. 2009
18	AFI11	Anoplopoma fimbria	1	52°C	F: GTT-TCA-CTC-CTA-CTG-GTC-TAG-G R: ATG-ACA-GCA-ACG-AGA-TGA-TTG	McCraney,W.T., Saski,C.A. and Guyon,J.R., 2012
19	AFIM X	Anoplopoma fimbria	1	52°C	F: TGC-TAA-ATG-TGG-GAT-GTG-AGT-T R: CTA-GGT-TTA-TGT-TTT-CAG-TGA-TGC	Messmer,A., Rondeau,E., Sanderson,D., Cooper,G., Leong,J., Campbell,B., Koop,B.F. 2009
20	AFI Y	Anoplopoma fimbria	1	52°C	F: ACC-AGC-TTC-ATA-GGA-CCG-TTT-A R: GAA-GTG-AGC-TCA-ACA-TAC-CAA-GAG	Messmer,A., Rondeau,E., Sanderson,D., Cooper,G., Leong,J., Campbell,B., Koop,B.F. 2009
21	Afim003	Anoplopoma fimbria	1	52°C	F: CTT-TGG-TGG-CCA-GAA-GAG-AT R: TTG-TTG-AGT-TTG-TTC-CAC-TGA-A	Messmer,A., Rondeau,E., Sanderson,D., Cooper,G., Leong,J., Campbell,B., Koop,B.F. 2009
22	AFI8	Anoplopoma fimbria	1	52°C	F: ACC-AGG-AGC-CTT-GAC-TGA-GTT R: CGC-GAA-AAG-TCT-GGA-ACA-GTA	McCraney,W.T., Saski,C.A. and Guyon,J.R., 2012

Table 3. PCR Programme №1.

Step	T, °C	Duration	Parameters
1	94	1 min	
2	94	1 min	
3	50 (52, 53, 56, 58, 60)	30 sec	
4	72	40 sec	
5	repeated 2-4		35 cycles
6	72	2 min	

5 samples, randomly selected from each from four sites (Table 1) (in total 20 samples) were analyzed. Polymorphism level between different sites in accordance of small numbers of samples

from each site which were analyzed didn't compare. At this stage common level of new analyzed jack mackerel's loci polymorphism was estimated. 20 samples for each from 22 new loci at specified temperatures were tested individually. Altogether 1060 locus-tests were conducted.

Table 4. PCR Programme №2.

Step	T, °C	Duration	Parameters
1	94	1 min	
2	94	1 min	
3	48 (50, 52, 54)	40 sec	
4	72	40 sec	
5	repeated 2-4		35 cycles
6	72	2 min	

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, 3). 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).

PCR product of jack mackerel was not obtained for 16 of 22 loci tested during this study. Reasons for this result could be that selected primer cites in jack mackerel microsatellites were different from original species.

Amplification of the other 6 loci was successful. 4 loci were polymorphic (Omy 1011, Ssa 419, OMM 1070, Gmo G18) and 2 - monomorphic (OMM 1037 *, Gmo 8). Loci Omy 1011 and Ssa 419 could be included in further study. The selection criteria for these loci, in addition to polymorphism, were also reliable amplification and size of the amplification products, suitable for analysis in acrylamide gel. Using loci OMM 1070, Gmo G18 as jack mackerel populations markers needs further studies including development of amplification technique.

In 2012-2013 it is also planned to sort out from the National Center for Biotechnology Information (NCBI) data base the microsatellite loci detected in phylogenetically close to jack mackerel species and to test them.