Achievements in the Life Sciences 8 (2014) 10–15

Contents lists available at ScienceDirect

Achievements in the Life Sciences

journal homepage: www.elsevier.com/locate/als

Genetic Markers for Studying the Current Distribution Area and Population Structure of the Sable *Martes Zibellina* L.

Oxana N. Zhigileva [a,*], Sergey N. Kashtanov [b], Inna M. Golovacheva [a]

[a] *Tyumen State University, 10 Semakova Street, Tyumen 625003, Russian Federation*
[b] *Vavilov Institute of General Genetics RAS, 3 Gubkina Street, Moscow 119333, Russian Federation*

ARTICLE INFO

Available online 11 November 2014

Keywords:
Martes zibellina
Martes martes
Hybrids
mtDNA
Haplotypes

ABSTRACT

Three types of genetic markers (restriction fragments of cytochrome *b* mtDNA gene, SSR and ISSR) were proposed for the study of genetic variability in the sable *Martes zibellina* within its geographic range. mtDNA haplotypes of different subspecies of the sable were described. Haplotypes of the eastern sable *Martes zibellina princeps*, which was introduced to Tyumen region in the 20th century, are rare in the gene pool of the modern sable populations of West Siberia. Haplotype diversity in the West Siberian sable *M. z. zibellina* is high due to introgressive hybridisation with the pine marten *Martes martes*. Nuclear genetic markers of *M. zibellina* × *M. martes* hybrids are more similar to the sable than to the pine marten.

© 2014 Published by Elsevier B.V. on behalf of Far Eastern Federal University.

Introduction

The sable is a zoological species with an interesting history. As one of the most valued fur-bearing animals, it was hunted for commercial uses and consequently was almost completely extirpated. In addition, the continuous vast habitat of the species has disintegrated into several isolated ones. As a result of massive reacclimatisation works carried out in the early and mid-20th century, the sable population and its area was revived (Acclimatisation, 1973; Monakhov, 1995; Bobrov et al., 2008).

Demonstrating a significant ecological flexibility and morphological variability, sables possess a complicated intraspecific structure and include several subspecies, although their number and distinguishing characteristics are the subjects of scientific debates (Pavlinov and Rossolimo, 1979). Because the valuable oriental sable subspecies (Barguzin) was used for the recuperation of the extinct populations due to its fur, the current population structure was disrupted. The acclimatised populations show some alterations in phenomenological appearance whose genetic nature is unknown (Ranyuk and Monakhov, 2011). Furthermore, migrations caused by nutritive base changes have always been common to sables. In the Ural region and West Siberia, where it dwells sympatrically with the closely related pine marten, interspecific hybridisation takes place (Rozhnov et al., 2010; Zhigileva et al., 2014).

This species, being of a high value and, at the same time, requiring population monitoring, serves as a good case to study microevolution processes in the circumstances of population depression, reintroduction and introgressive hybridisation. These tasks require the elaboration of genetic markers of the species, which is the objective of the present paper.

* Corresponding author.
E-mail address: zhigileva@mail.ru (O.N. Zhigileva).
Peer review under responsibility of Far Eastern Federal University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.als.2014.06.004
2078-1520/© 2014 Published by Elsevier B.V. on behalf of Far Eastern Federal University.

Table 1
Complex haplotype frequencies of the cytochrome *b* mtDNA gene of the Martes genus in different parts of the distribution area.

Line	Complex haplotype	Ural	West Siberia			Eastern Siberia	Far East
		Sable	Martes	Kidus	Sable	Sable	Sable
Z28	AAAA	0	0	0	0.030	0.38	0.41
Z30	BBBB	0	0.1	0.326	0.619	0.62	0.54
Z31	BABB	0	0	0.087	0.227	0	0.05
AK23	AACA	0	0.1	0.326	0.052	0	0
AK29	BBAB	0	0.45	0.022	0	0	0
Z5	CBAB	0	0.35	0.196	0.031	0	0
UC1	AABA	1.0	0	0	0	0	0
AC27	CBBB	0	0	0	0.041	0	0
AK55	CBCB	0	0	0.043	0	0	0
Sample size (*n*)		3	20	46	97	21	37

Material and Methods

Sable carcasses *Martes zibellina* L. were used as material for the research. They were obtained by hunters during hunting seasons between 2008 and 2012 in West Siberia (Tobolsk, Vagaisk, Uvatsk, Nefteyugansk, Soviet, Nizhnetavdinsk and Tyumen areas in the Tyumen region). Tissue samples from the pine marten *Martes martes* L. and "kidus" (sable and pine marten hybrid) were used for comparison. They were hunted in the above-mentioned areas, the Isetsk and Yalutorovsk areas of the Tyumen region and the Omsk region. Some materials were pieces of sable skins that were hunted in Eastern Siberia and the Far East (Yakutia, Sayany mountains, Amur River basin, Sakhalin Island, Kamchatka Peninsula). A total of 224 animal units were used as samples.

Three methods were applied to identify DNA polymorphisms — restriction analysis of the cytochrome *b* of mitochondrial DNA (mtDNA) gene fragment, Inter Simple Sequence Repeat Polymerase Chain Reaction (ISSR-PCR) and analysis of the variability of the Simple Sequence Repeat (SSR, or microsatellite) gene locus. The total DNA was extracted from cardiac muscle tissue fixed in 70% ethanol and from skin samples using the Diatom DNA Prep100 kit for DNA extraction (Laboratoria Izogen Ltd., Moscow, Russia).

The restriction analysis of the 1300 bps cytochrome *b* mtDNA gene fragment utilised primer sequences from the work of Balmysheva and Solovenchuk (1999) and the endonucleases Hae III, BstN I, Taq I, Rsa I. The choice of restriction endonucleases was stipulated by the presence of the respective recognition sites in the analysed section of both the sable and marten mitochondrial genomes (Koepfli et al., 2008). Touchdown PCR of the cytochrome *b* gene of a mtDNA fragment was carried out in a 20 mcl μL reaction mix containing the IQ supermix (Bio-Rad Ltd., USA), 3 μL of total DNA and 2.5 μL of each of the primers on the amplifier DNA Engine Dyad ® Chromo4 (Bio-Rad Ltd., USA) in the following mode: 94 °C–5 min, then 33 cycles of 94 °C–1 min, 51 °C–1 min, 72 °C–1 min 45 s; 72 °C–2 min. Electrophoretic separation of restriction fragments was performed by a 2.5% agarose gel. Fragment length was determined using the GeneRuler™ DNA Ladder mix DNA molecular weight marker (Fermentas Ltd., Lithuania).

Touchdown PCR of the microsatellite *Elu*1 utilised primers and the amplification mode described by Kretschmer et al. (2009). Microsatellite fractions were analysed with a 6% polyacrylamide gel. To determine the size of the alleles, the plasmid pBR322 treated with the restriction enzyme Hpa II (Fermentas Ltd., Lithuania) was used as a DNA molecular weight marker. Visualisation of the PCR products and restriction endonuclease were carried out through staining of the gels in ethidium bromide solution and observation under ultraviolet light, and digital images were obtained with a Kodak 1D gel documentation system.

Five primers, $(AG)_8C$, $(GT)_8C$, $(TC)_8C$, $(AC)_8T$, $(TG)_8A$, were used for ISSR-PCR. Sequence amplification limited by simple repeats was performed in a 25 μL reaction mix containing PCR buffer (0.01 M Tris–HCl, 0.05 M KCl, 0.1% Triton X-100), 4 mM $MgCl_2$, 0.2 mM each dNTP, 1 μL solution total DNA, 2.5 mM primer and 0.2 units/μL Taq polymerase (Fermentas Ltd., Lithuania) on a Chromo4 thermocycler (Bio-Rad Ltd., USA) in the following regime: 94 °C–7 min; 40 cycles of 94 °C–30 s, 52 °C (for primers $(AC)_8T$, $(TG)_8A$) or 56 °C (for primers $(AG)_8C$, $(GT)_8C$, $(TC)_8C$)–45 s, 72 °C–2 min; 72 °C–7 min. ISSR-PCR fragment analysis was performed with a 2% agarose gel. Calculation of population genetic parameters and construction of dendrograms were carried out using POPGEN software (Yeh et al., 1999).

Table 2
Complex haplotype frequencies of the cytochrome *b* mtDNA gene of the various sable subspecies.

Sable subspecies	*n*	Z28	Z30	Z31	AK23	Z5	AC27
West Siberia sable *M. z. zibellina*	97	0.030	0.619	0.227	0.052	0.031	0.041
Yenisey sable *M. z. yenisejensis*	9	0.222	0.778	0	0	0	0
Sayan sable *M. z. sajanensis*	12	0.500	0.500	0	0	0	0
Barguzin sable *M. z. princeps*	13	0.308	0.615	0.077	0	0	0
Kamchatka sable *M. z. kamtschadalica*	12	1.000	0	0	0	0	0
Sakhalin sable *M. z. sahalinensis*	12	0	1.000	0	0	0	0

Download English Version:

https://daneshyari.com/en/article/1351103

Download Persian Version:

https://daneshyari.com/article/1351103

Daneshyari.com