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Analysis of Mitochondrial DNA Variation in the Population of Oroks

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Abstract—Mitochondrial DNA (mtDNA) variation was studied in population of Oroks ($N = 61$), the indigenous inhabitants of Eastern Siberia. Most of the mtDNA types examined fell into five haplogroups (C, D, G, M10, and Y) typical of Eastern Eurasian populations. For three haplogroups (D, C, and M10), the founder effect was established. In one individual, a unique lineage belonging to haplogroup HV and typical of Caucasoids was detected.

INTRODUCTION

Specific features of mtDNA, including maternal inheritance, lack of recombinations, and high variation make it a useful tool for evolutionary, population, and medical genetic studies [1]. At present, the nomenclature of mtDNA types for the populations is based on the synthesis of the control-region (hypervariable segments I and II) and coding-region polymorphism data [2–7]. All mtDNA lineages identified in Europe, Asia, and America are considered to be the progeny of one cluster, L3n (N). The complete set of Asian mtDNA lineages contains two macroclusters, M and N [3]. High frequency and diversity of cluster M lineages in populations of Asia [8, 9] along with their low incidence in Europe favor the hypothesis that Asia was populated as a result of population migration from Eastern Africa [10]. About 50% of mtDNA lineages found in the indigenous populations of Eastern Eurasia belong to cluster M, which is in turn, subdivided into haplogroups (D, G, M7, M8, M9, M10, and others). Supercluster N contains three large branches: (1) branch A; (2) new branch N9, distinguished by the transition in nucleotide position 5479 bp and including haplogroups Y and N9a; and (3) branch R, which is a supercluster of mtDNA lineages widespread all over Eurasia [3]. Almost all Eastern Asian lineages of cluster R belong to two large haplogroups, B and F.

Population diversity of Northern, Eastern, and Central Asia along with the complexity of the ethnic history and wide settling range of these populations are extremely interesting for studying the population genetic structure. Variation of mtDNA was evaluated in many ethnic groups of Siberia (Eskimos, Chukchi, Koryaks, Evens, Evenks, Altaians, Tuvinians, Yakuts, Buryats, and others) [11–17]. Intensive studies of

mtDNA polymorphism were carried out among the indigenous inhabitants of Siberia and the Far East with respect to the problem on the prehistoric peopling of the Americas [12, 14, 18]. The gene pools of the populations examined were characterized by the different ratios between Mongoloid (M, A, B, F) and Caucasoid mtDNA lineages (H, U, T, J, K). For each ethnic group, specific mitochondrial haplogroup frequency distribution pattern was established. For instance, mtDNA types belonging to haplogroups A, B, C, and D typical of the indigenous populations of America were found in all Southern Siberian populations and prevailed in the populations of Northeast and East Asia [12–17].

A number of ethnic territorial groups of North Asia, Oroks in particular, remain poorly studied in population genetic respect. Oroks are the least represented ethnic group in Russian Federation, which occupies the small part of northeastern Sakhalin territory [19]. According to the survey by S. I. Bruk (1986), the total population number of Oroks was about 200 [20]. Linguistically, Oroks belong to the southern, or Manchurian subgroup of Tunguso–Manchurian populations, which also include some other small ethnic populations from lower Amur River, Primorye, and Sakhalin (Nanais, Ulchi, Orochis, Negidalts, and Udegeis) [21]. Unlike most of the Manchurian native populations living on the territories of the Amur River basin and Primorye, who were traditionally fishermen, Oroks from Sakhalin since ancient times are deer-breeders. Most of the cultural traditions specific of this ethnic group point to its continental origin. It is suggested that that they have migrated to Sakhalin in the 17th century from the territories of the Amur River basin [19]. Anthropologically, Oroks are characterized by a complex of morphological traits typical of the Baikal type, which bring

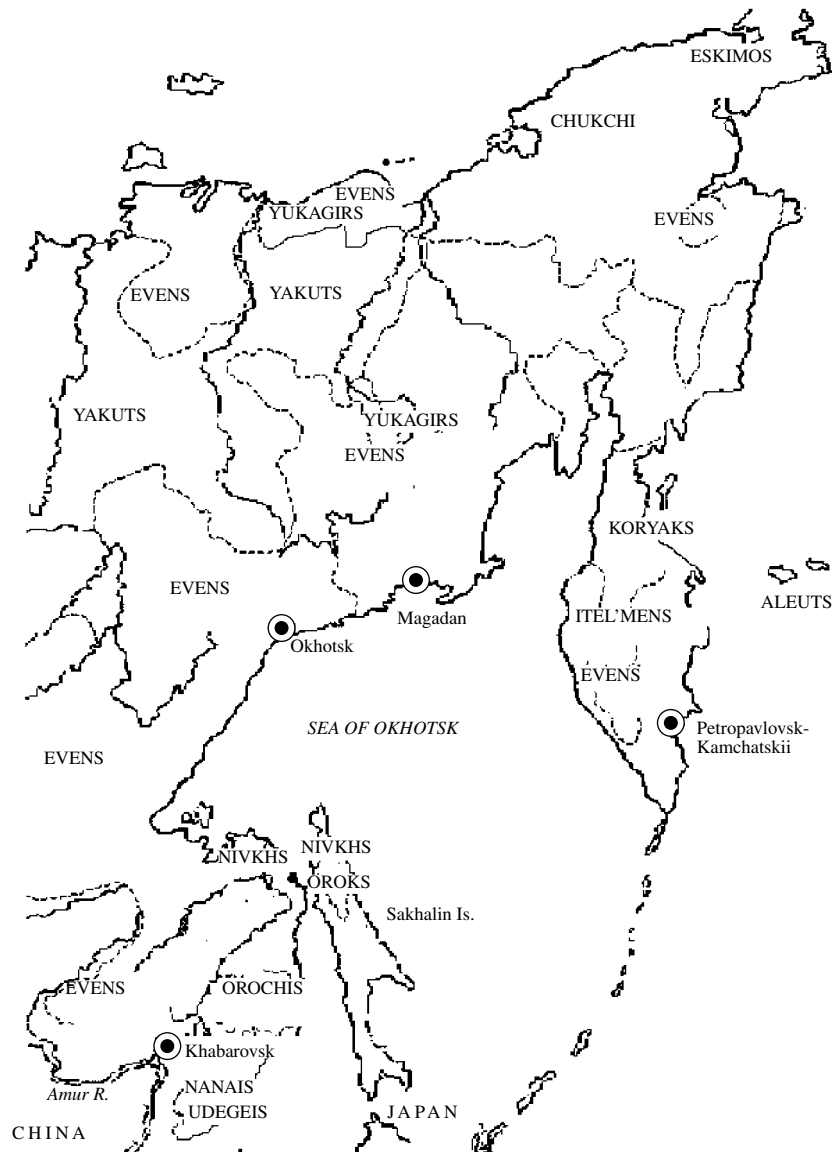


Fig. 1. Geographic distribution of Eastern Siberian and Far Eastern populations.

them close to Evens, Northern Evenks, Yukagirs, and Negidalts.

The present study was focused on the characterization of the mtDNA lineage diversity in Oroks, based on the sequence data for the HVS-I of the main noncoding region and restriction polymorphism of mtDNA.

MATERIALS AND METHODS

The samples for population genetic analysis were collected during expeditions to the Far East in 1998 (Fig. 1). Blood specimens were collected from adults after medical examination and obtaining the informed consent on the population genetic survey. Ethnic affiliation of the individuals was determined through ques-

tionnaire, which included the data on the ethnicity of the relatives for three generations. The DNA samples from Sakhalin Oroks ($N = 61$) were used.

MtDNA was isolated from peripheral blood by use of standard procedure of phenol–chloroform extraction. Sequencing of hypervariable segment I (HVS-I) of the mtDNA control region was carried out between the nucleotide positions 16024–16400 using ABI 377 DNA Sequencer (Perkin–Elmer) and DYEnamic™ ET terminator sequencing premix kit (Amersham Pharmacia Biotech).

To determine mtDNA haplogroups, RFLP analysis was performed at the restriction endonuclease recognition sites as follows: 73 (73GA) – *Alw44I*; 5176 (5178CA) –

Table 1. MtDNA lineages in Oroks ($N = 61$)

No.	Haplogroups	HVS-I motif	N
1	C	223-298-325-327-356*	7
2	D	148-223-362	1
3	D	148-223-263-362	2
4	D	223-274-362-368	4
5	D	129-223-274-291-311-362	29
6	D	129-223-274-287-291-311-362	1
7	D	93-129-223-274-291-311-362	1
9	D	93-223-232-261-290-362	3
8	D5	92-172-182-183-186-189-223-266-362	1
10	M10	93-129-223-311	5
11	G	93-207-223-399	2
12	G	93-207-223-244-399	1
13	Y	126-183-189-231-266-292	2
14	Y	126-183-189-231-262G-266-294	1
15	HV	111-311	1

* The figures are given with subtraction of 16 000, i.e., 223 instead of 16223.

AluI; 7025 (7028CT) – *AluI*; 10394 (10398AG) + *DdeI*; 10397 (10400CT) + *AluI*; 12704 (12705CT) – *MboII*; 13262 (13263AG) + *AluI*; 5416 (5417GA) + *TasI*; 7598 (7598GA) – *HhaI*; 4830 (4833AG) + *HaeII*; and 14766 (14766TC) + *MseI*. In brackets are transitions or transversions, responsible for the corresponding restriction site gain or loss. Mutations in mtDNA are numbered in accordance with the so-called Cambridge Reference Sequence (CRS) [22]. For exact definition of phylogenetic relationships between the haplotypes, a median network was constructed based on the combination of the data on the coding region and HVS-I polymorphism in total Orok sample.

RESULTS AND DISCUSSION

Analysis of the Orok mtDNA lineages enabled their attribution to haplogroups M10, D, G, C, Y, and HV. Twelve out of fifteen mtDNA haplotypes determined belonged to macrocluster M (Table 1), which was distinguished by the presence of two closely located restriction sites, +*DdeI* 10394 (10398AG) and +*AluI* 10397 (10400CT). The exception was one sample, belonging to haplogroup D5. Macrocluster M includes haplogroups M8, M9, D, and G, which are characterized by the presence of specific restriction sites within the mtDNA coding region along with the presence of specific HVS-I motifs [3]. Haplogroups D and G carry cytosine in HVS-I nucleotide position 16362. Sister haplogroups C and Z originate from the common node M8, distinguished for the transitions at the sites 16298, 4715, and 8584, along with transversions at nucleotide positions 7196 and 15487 [23].

Analysis of the geographic distribution of haplogroup D (–5176 *AluI*; HVS-I motif 16223–16362)

among the populations of Northeastern and Eastern Asia demonstrated strict correlation between its frequency and latitude (Table 2). The lineages belonging to this haplogroup were detected in the populations of Central Asia and Siberia [24]; they were also found in Southeast Asia at a very low frequency. More than one-third of mtDNA lineages described in Nganasans and Yukahirs belong to haplogroup D [9], which was also found in American Indians. In the present study, haplogroup D was represented by eight HVS-I types (Fig. 2). The frequency of haplogroup D in Oroks (69%) was the highest among the Eurasian populations, sharply contrasting with its low frequency in Nivkhs, also inhabiting the Sakhalin Island (4%), and those living on the continent (28%). Lineage 16129-16223-16274-16291-16311-16362 accounting for 47.5% of mtDNA in Oroks was the most frequent in the sample examined. Earlier motif 16223–16274–16362 was detected in Mongols (8.8%) and Uigurs [25]. MtDNA lineage 16223-16274-16291-16362 was found in Kyrgyzia [24]. This lineage, represented as no. 9, is determined by the substitutions at nucleotide positions 16093-16223-16232-16261-16290-16362. This motif, however, without an additional mutation at nucleotide position 16261, was earlier found in Nivkhs, Chinese, and with the frequency of 17.6% in Mongols [25]. Four Orok mtDNA samples carry substitutions at the sites 16223-16274-16362-16368. Similar haplotype was found in Kyrgyzes (Central Asia) [24]. In one case, mtDNA type belonging to haplogroup D5, which is defined by the presence of a transition at the site 16189, along with the loss of the RFLP marker for cluster M (caused by a transition at the site 10397) was determined. Haplogroup D5 is most frequent in Southern

Table 2. Frequency of mtDNA haplogroups in the populations of Eastern Eurasia

Population	M*	C	Z	D	G	E	A	B	F	Y	N, R*	N
Oroks	13.1	11.5	0.0	68.9	0.0	0.0	0.0	0.0	0.0	4.9	1.6	61
Nivkhs ¹	1.8	0.0	0.0	3.6	25.5	0.0	0.0	0.0	0.0	67.3	1.8	55
Nivkhs [9]	1.8	0.0	0.0	28.1	5.3	0.0	0.0	0.0	0.0	64.9	0.0	57
Udegeis [9]	44.4	17.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.9	28.9	45
Evenks [9]	0.0	84.3	0.0	9.8	0.0	0.0	3.9	0.0	2.0	0.0	0.0	51
Chukchi [12]	0.0	10.6	0.0	12.1	9.1	0.0	68.2	0.0	0.0	0.0	0.0	66
Koryaks [11]	0.0	36.1	5.8	1.3	41.9	0.0	5.2	0.0	0.0	9.7	0.0	155
Itel'mens [11]	0.0	14.9	6.4	0.0	68.1	0.0	6.4	0.0	0.0	4.3	0.0	47
Buryats [13]	22.5	20.0	0.0	25.0	0.0	10.0	5.0	5.0	5.0	0.0	7.5	40
Tuvinians [13]	5.6	36.1	0.0	13.9	0.0	8.3	5.6	13.9	8.3	0.0	5.6	36
Mongols [24]	13.6	14.6	3.9	24.3	n.d.	2.9	3.9	9.7	8.7	1.9	16.5	103

Note: ¹ Our unpublished results; n.d., no data.

China, while in Central Asia and Siberia it is either absent, or very rare [24–26].

Haplogroup C (+13262 *AluI*; HVS-I motif 16223-16298-16327) prevails in the populations of Northern Asia. Its geographical distribution ranges southward from Kamchatka to Japan and eastward from the eastern borders of Europe to the Far East. Maximum frequencies of haplogroup C were detected in Evenks (84.3%) and Yukagirs (59.3%) [9]. Haplogroup C is one of four mtDNA haplogroups found in Native Americans [8, 18], which are distinguished by the transition at nucleotide position 16325 [27]. The haplogroup is rare in the populations of Asia [24, 25]. The substitution at the nucleotide position mentioned was detected in Oroks. Haplogroup C is the second most frequent in the population examined, albeit is represented there by only one lineage. The HVS-I motif 16223-16298-16325-16327-16356 was earlier described in the populations of Mongols and Altaians [25].

Similarly to haplogroups D and M9, haplogroup G diverged from the common node M* and carries a substitution at the site 16362. Phylogenetic analysis subdivides haplogroup G into subclusters as follows. G1 is typical of the populations of Northeastern Siberia, where it reaches maximum frequency among Koryaks (42%) and Itel'mens (68%). G2a is most frequent in Central Asia (8.8%) and in Yakuts (4.1%). In the population sample examined mtDNAs from three specimens belonged to haplogroup G. The HVS-I motif 16093-16207-16223 was also detected in Nivkhs. It seems likely that the lineages determined fall into subgroup G1, since they have a reverse mutation at the site 16362. To establish the exact positions of these lineages on the phylogenetic tree, additional investigations are required. The HVS-I motif 16093-16129-16223-16311, detected in five individuals belongs to haplogroup M10.

Haplogroup Y (+7933 *MboI*, –8391 *HaeIII*, +10394 *DdeI*; HVS-I motif 16126-16231-16266), the deriva-

tive of cluster N9, was first detected in Northeastern Asia [11]. In Ainu (Hokkaido Island) its frequency reaches 20%. This haplogroup was also found in Kamchatka, Sakhalin, and Korea, but it is absent from the majority of Siberian populations [9, 11, 28, 29], unlike its sister haplogroup N9a, which, however is distributed among all Eastern Asian populations at a low frequency. It is suggested that haplogroup Y appeared among the indigenous inhabitants of the Amur River basin [11]. The spread of this haplogroup westward was associated with the ethnic groups belonging to the Altaic linguistic family [11]. Interestingly, in Oroks the lineages belonging to this cluster are found at frequencies about 5%, which are much lower than those in the closest neighboring population of Nivkhs (approximately 66%). The presence of these lineages suggests the continental origin of the indigenous population examined. It seems likely that Oroks immigrated to Sakhalin from the Amur River basin [19]. On the other hand, mtDNA haplogroup Y lineages distribution patterns in Oroks (4.9%), Udegeis (8.9%), Nivkhs (64.9%), and Ainu (19.6%) point to common genetic basis of these populations.

In the sample of Oroks examined, one unique haplotype belonging to haplogroup HV was detected. This cluster is absolutely not typical of Eastern Eurasia. Based on the phylogenetic analysis of Western Eurasian mtDNA lineages, it was established that supercluster N has one large branch represented by cluster R, which was defined by the substitutions at nucleotide positions 12705 and 16223 [2, 4]. Haplogroups H, V, HV, J, T, and U form this cluster. Haplogroups H, V, and HV have a common ancestor, which comes out from internal node HV*. Most of the derivatives of this cluster are found in the Near East, which is considered to be the place of origin of this haplogroup. Haplogroup HV is found in the Caucasus (Georgia, Armenia, and Osetia) at the frequency of about 8%, and at a lower frequency, in Europe, India, and Central Asia [24, 30]. The

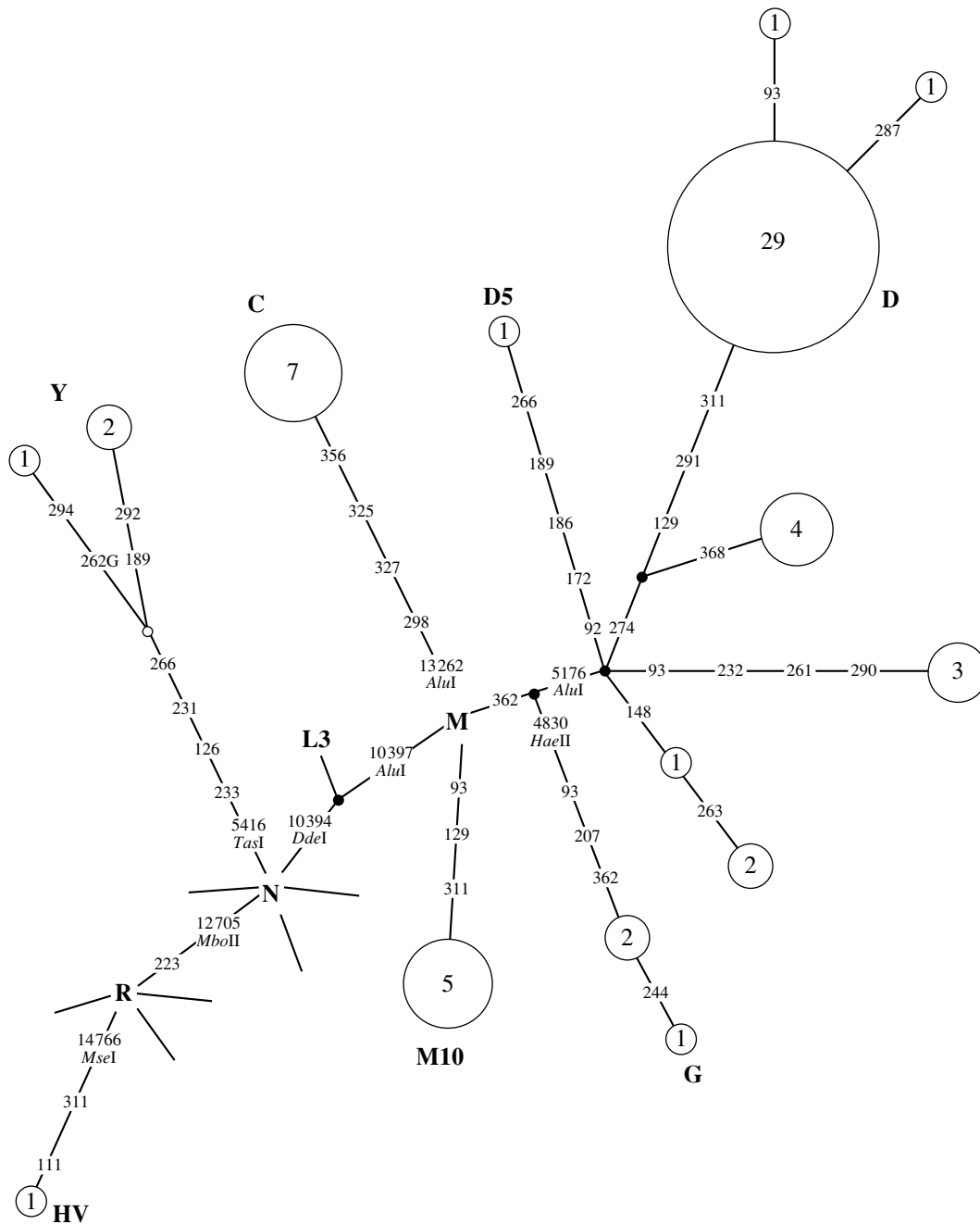


Fig. 2. Phylogenetic tree of mtDNA lineages in Oroks. The size of each circle is proportional to the haplotype frequency. The numerals in the circles designate the numbers of the individuals in the population carrying the given haplotype. Mutations were determined relative to the revised Cambridge Reference Sequence and are presented as numerals defining mutations at nucleotide positions from 16024 to 16400, i.e., 16223 is designated as 223. For each cluster, restriction diagnostic sites within mtDNA coding region are indicated.

presence of haplogroup HV in Oroks may be explained by contacts with the populations having migrated to the Far East from the European part of the continent.

Thus, analysis of mtDNA lineages in Oroks showed that this ethnic group is characterized by a unique set of mtDNA types, which distinguish this ethnic group from the Siberian populations, which are close to it linguistically and geographically. For haplogroups D, C, and M10, the founder effect was shown. MtDNA lineages

distribution patterns and the level of their genetic diversity in Oroks point to the processes of genetic drift, which have a strong influence on small populations.

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