

Electrophoretic identification of allozyme variation in natural population of ticks *Ixodes persulcatus* Schulze (Acari: Ixodidae) in Irkutsk suburbs*

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ABSTRACT

Allozyme structure of the tick population *Ixodes persulcatus* coded by nine enzyme gene loci has been studied using two subsamples of ticks from Irkutsk suburban areas. Nine loci out of fourteen were polymorphic in ticks of Baikal highway population, whereas five loci were polymorphic in ticks sampled

from Goloustnoye highway. High level of intra-population heterogeneity of ticks has been revealed. No distinction between two populations from different nidi of infection has been recorded. It was probably due to the high rate of gene migration between the studied populations and to their high genetic similarity.

INTRODUCTION

In the suburbs of Irkutsk there are nidi of mixed tick-borne infections such as tick-borne encephalitis, tick-borne borelioses and ehrlichioses (Alekseev et al. 2001; Nikitin and Antonova 2005). These infections are widely spread in the forest zone of Eurasia from the Atlantic to the Pacific Oceans. From west to east, the principal carrier – a forest *Ixodes ricinus* tick is replaced by a taiga *I. persulcatus* tick. The species composition of vertebrates, tick hosts, also changes, whereas the peculiar characteristics of disease agents remain unchanged (Balashov 2010).

General principles of population organization in ticks of the genus *Ixodes* are widely studied. Total abundance of ticks in a nidus, percentage of infected individuals, abundance of active specimens seeking their hosts, life-cycle of hungry ticks, chances for diseases agents to meet vertebrates susceptible to pathogens, and contact of ticks with other infection agents – all these factors play a significant role in the population structure of ticks (Balashov 2010).

Heterogeneity of specimens in tick populations is of interest, as phenotypically and genetically variable ticks

possess different infection rate and reaction to the presence of agents in infected specimens and provide preservation of pathogen genotype diversity. Heterogeneity also causes the substitution of some agents for the others (Alekseev and Dubinina 1997, 2007; Alekseev et al. 2007; Balashov 2010; Korotkov 2005; Podbornov et al. 2007; Semenov et al. 2001).

Phenotypic variability the *Ixodes* ticks is well studied (Alekseev and Dubinina 1993; Estrada-Pena et al. 1996; Kozlova et al. 2008a, b), but there are few data about their genetic variability, mostly based on allozyme data. High intrapopulation and low interpopulation genetic variability in loci *a-Gpdh** and *Pgm** were registered in Irish, Swedish and Swiss populations of *I. ricinus* (Delaye et al. 1997; Healy 1979a, b) and in the loci *a-Gpdh** and *Mdh** in the three populations of forest ticks of Slovenia (Radulovic et al. 2006). The studies of genetic structure of *I. persulcatus* are fewer than those of the other species of ticks (Balmelli and Piffaretti 1996; Eisen and Lane 2002; Fukunaga et al. 2000; Meeu et al. 2002).

It is supposed that biotic and abiotic factors significantly affect tick genotypes. The relationship between locomotive activity of *I. ricinus* and its *a-Gpdh* isoenzymes was determined (Healy 1979a), and seasonal dynamics of

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occurrence of different *Mdh**-genotypes of these populations of ticks in Denmark were studied (Jensen et al. 1999).

Borreliae infection is related to the polymorphism on loci *Mdh** and *a-Gpdh** of *I. ricinus* in Slovenia (Radulovic et al. 2006) and on locus *Mdh** of *I. persulcatus* in the suburb of St.-Petersburg (Semenov et al. 2001). *Mdh** genotype changes of *I. ricinus* depended on age or on solar activity registered on the day of observation (Jensen et al. 1999). Anthropogenic influence causes changes in morphology of ticks which in turn change physiology of carriers (Alekseev et al. 2006). This in turn resulted in a change of the occurrence frequency of dominant strains of tick-borne encephalitis virus (Alekseev et al. 2007). The tick-borne encephalitis virus stimulates mobile activity of ticks (Alekseev et al. 1988). On the other hand, mobile activity is suppressed in borreliae-infected *I. persulcatus* (Alekseev and Dubinina 2000).

Dynamics of borreliosis disease correlates with the abundance of bloodsucking taiga females with altered morphology (Zharkov et al. 2000).

The studies on heterogeneity of *I. persulcatus* population are very urgent, as the abundance of ticks, their pathogen infection and the number of tick-borne encephalitis cases are higher in the Irkutsk Oblast than on average in Russia (Borisov et al. 2002; Zlobin and Gorin 1996). The purpose of the present study was to assess genetic variability in natural populations of taiga ticks in nidi with different anthropogenic impact.

MATERIAL AND METHODS

Ticks were collected in woodlands along the Baikal highway (BH) and Goloustnoye highways (GH) of the Irkutsk Oblast in 2006-2008 (Figure 1). The length of sampling site was over 500m.

The site of tick sampling along the Baikal highway (127 specimens) was located 47km off Irkutsk and 2km off the Irkutsk Reservoir (51°59' N, 104°40' E) along the low bank (about 100m altitude) of the Angara River. This is a heavy traffic highway with a great number of summer cottages, recreation centers and hotels on both sides of the road.

The site of tick sampling along the Goloustnoye highway (13 specimens) was located in the vicinity of the village Dobrolet (52°15' 07' N, 104°49' 51' N) 33km off Irkutsk and goes through the territory of the Primorsky Ridge at 900-1200m altitude (Atutova 2008). The anthropogenic impact on GH is less. Ticks inhabit mainly fallen trees along the road. The direct distance between BH and GH is about 40 km.

The ticks were taken by dragging the white flag between 9 a.m. and 12 a.m. during their seasonal peak activity from early May to early June. Only adult specimens were selected for the analysis. Ticks were transported to the laboratory in damp gauze, frozen in plastic tubes at -18°C. Each tick was analyzed individually. All specimens were homogenized in 100µl 0.05M of Tris-HCL buffer (pH 7.2) with addition of 1% Triton X100, 40% of sucrose and β-merkaptoetanol (Jensen et al. 1999), centrifuged for 30min (10,000 rpm) to remove insoluble material.

Electrophoretic analysis was carried out in vertical blocks of polyacrylamide gel using standard methods (Davis 1964; Peacock et al. 1965). Two electrode buffer systems, such as TRIS-GLYCINE and TRIS-EDTA-BORIC ACID, were used to separate proteins. Nine enzyme systems (Table 1) were studied. Protein detection was performed according to Aebersold et al. (1987) and Shaw and Prasad (1970). Loci were designated according to recommendations of Shaklee et al. (1990). Revealed genetic variations by most loci were interpreted according to schemes known for ticks (Healy et al. 2004; Hilburn and Sattler 1986; Semenov et al. 2001).

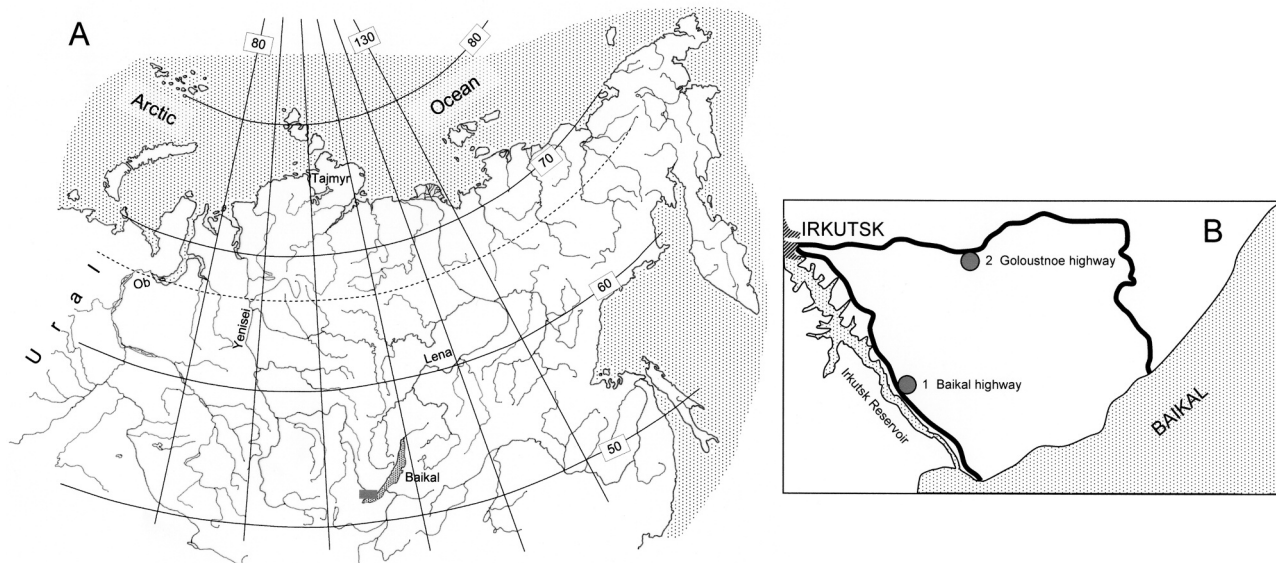


Figure 1. Map of Siberia (A) with Irkutsk region study area and sampling sites (B) of ticks, *Ixodes persulcatus*.

Table 1. The investigated enzyme systems and allele frequencies in polymorphic loci of ticks (*Ixodes persulcatus*).

Enzyme	Buffer	Locus	Allele	Allele frequencies	
				Baikal highway (BH)	Goloustnoye highway (GH)
Aconitase (4.2.1.3)	A	<i>AH-1*</i>	100	0.77 (19)	0.90 (5)
			150	0.23	0.10
Diaphorase (1.6.3.1)	A	<i>DIA-1*</i>	100	0.85 (30)	0.80 (8)
			80	0.15	0.20
Carbonic anhydrase (4.2.1.1)	A	<i>CA-1*</i>	100	0.87 (40)	0.88 (13)
			105	0.13	0.12
		<i>CA-2*</i>	100	1.00 (40)	1.00 (13)
		<i>CA-3*</i>	100	1.00 (40)	1.00 (13)
Creatine kinase (2.7.3.2)	A, B	<i>CK*</i>	100	1.00 (40)	1.00 (13)
Lactate dehydrogenase (1.1.1.27)	B	<i>LDH*</i>	100	1.00 (40)	1.00 (13)
Malate dehydrogenase (NAD⁺, 1.1.1.37)	A	<i>MDH-1,2*</i>	100	0.85 (24) ^t	0.92 (6)
			80	0.15	0.08
Superoxide dismutase (1.15.1.1)	A	<i>SOD-1*</i>	100	0.82 (50)	1.00 (13)
			150	0.18	0.00
		<i>SOD-2*</i>	100	0.83 (50)	1 (13)
			150	0.17	0.00
Esterase (3.1.1.-)	A	<i>EST-1*</i>	100	1.00 (14)	1.00 (8)
		<i>EST-2*</i>	100	0.75 (14)	0.81 (8)
			150	0.25	0.19
Esterase D (3.1.-)	B	<i>ESTD*</i>	100	1.00 (20)	1.00 (13)
$\chi^2 = 1.344$					
P (%)				57.1	35.7
H_{exp} (%)				16.41±0.04	7.18±0.03
D_N				0.011	

Buffer: A - TRIS-GLYCINE (Davis 1964); B - Tris-EDTA-BORIC ACID (Peacock et al. 1965); chi-square test χ^2 - heterogeneity test; P - percentage of polymorphic loci; H_{exp} - mean heterozygosity; ^t - test of correspondence between observed and expected (Hardy-Weinberg) distribution of genotypes, significance level P<0.05; D_N - standard genetic distance. Number of investigated ticks are in brackets.

The analysis of allozyme variations and test for homogeneity of allele frequencies among samples were performed with BIOSYS-2 Program (Black 1997).

RESULTS

Nine studied enzyme systems were encoded by 14 loci, 8 of which were polymorphic in ticks sampled in BH and 5 in GH (Table 1). Phenotypes of studied loci corresponded to homological ones with the appropriate genetic control

described earlier for closely related species (Healy 1979a, b; Healy et al. 2004; Hilburn and Sattler 1986; Semenov et al. 2001). The character of phenotype distribution among isoloci *Mdh-1,2** made it possible to accept the model of two polymorphic loci with equal frequencies of corresponding alleles (Figure 2). The distribution of phenotypes in all isoloci of the study samples corresponded to the Hardy-Weinberg equilibrium except for *Mdh-1,2** in BH. A high level of variation was revealed in all polymorphic loci, as well as average heterozygosity and polymorphic loci (Table 1). The analysis of allele frequencies in polymorphic loci using the

test for homogeneity showed significant differences in loci *Sod-1** ($P < 0.05$) and *Sod-2** ($P < 0.05$) between BH and GH samples. Fixed alternative alleles were not recorded in studied tick samples. The data on allozyme analysis demonstrated high heterogeneity of tick samples collected from each (BH and GH) site, and no differences between BH and GH samples.

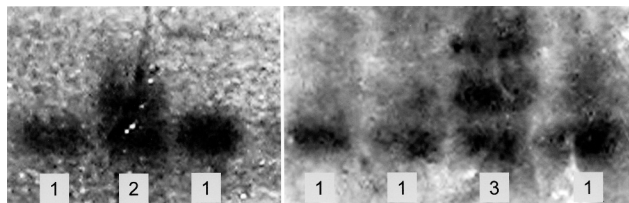


Figure 2. *Mdh** phenotypes of *Ixodes persulcatus*. 1 - 100/100/100/100, 2 - 100/100/100/80, 3 - 100/100/80/80.

DISCUSSION

The analysis of allele frequencies of polymorphic loci (Table 1) revealed low level of genetic variation between tick samples collected along BH and GH and significant heterogeneity within the samples. Similar results have been obtained on studies of biometric variation *I. persulcatus* populations of two geographically remote territories of the Irkutsk Oblast. A high level of phenotypic variability was recorded in the tick populations from the suburbs of Bratsk. However, no differences were determined between ticks of this area and those from the suburbs of Bolshiye Koty in the vicinity of Lake Baikal (Kozlova et al. 2008a, b). One of the possible reasons could be a high migration activity of rodents, the carriers-hosts of ticks which are the main reservoir of pathogens. Hilburn and Sattler (1986) confirm that the genetic variation within and between populations depends on mobility of a host, size of tick population and level of tick specificity for a host. Since the 1980-ies, the abundance of ticks has increased by 80 folds in the Irkutsk Oblast (Korotkov et al. 2007). In 2007, sampling provided 154 specimens per flag-km along GH, and in 2008 it was 162 specimens per flag-km along BH (Nikitin and Antonova 2005). Besides high level of tick abundance, possible reasons of the absence of genetic differences between these two samples may be high migration activity of tick carriers (rodents and birds), the absence of geographical barrier, and relative proximity of Baikal and Goloustnoye highways.

The analysis of 14 enzymes in 9 populations of *Amblyomma americanum* (L.) (family Ixodidae) of the South-East USA showed the absence of geographical structure of the species and its genetic heterogeneity. The low level of heterozygosity was detected in populations of this species by Hilburn and Sattler (1986). Low interpopulation variability was determined

in Irish and Swiss populations of *I. ricinus* (Delaye et al. 1997; Healy 1979a, b). The gene flow between *I. ricinus* populations whose specimens are transferred by the mobile vertebrates is considered to be the cause of the formation of panmictic areas (60-70km in diameter) (Delaye et al. 1997; Healy 1979a, b; Radulovic et al. 2006). The distance between BH and GH is about 40km which is much shorter than that between genetically differentiated populations of *I. ricinus* (Radulovic et al. 2006). However, significant genetic variability between Irish and Swedish coastal *I. ricinus* populations (Healy et al. 2004) are attributed to climatic differences between the island and coastal part of the continent.

It is possible that the influence of natural selection, genetic drift and migration on the state of natural tick populations may be much lower as compared to that biotic and abiotic factors. Some authors note that the intensity of infection by borreliae depends on gene groups (Semenov et al. 2001). The relationship was determined between *Mdh** genotypes and intensity of borreliae-infection (Semenov et al. 2001), geographical variability of *Mdh** genotypes and subtypes of tick-borne encephalitis virus (Alekseev et al. 2007). High intrapopulation heterogeneity of ticks from the two nidi is probably attributed to the fact that anthropurgic active nidi emerged in the suburbs of Irkutsk in which natural structure of tick populations changed: at present there is a great number of hosts and high rate of pathogen circulation.

Many authors attribute variability in tick populations to their pathogen infection. A number of factors, including biochemical characteristics of ticks, affect the level of specific relationship between bloodsucking ticks and disease agents (Podbornov 2004). The activity of various parasites changes the initial physiological, biochemical and other reactions of an invertebrate host-carrier to environmental factors. Different species of *Borrelia* change sensitivity of *I. persulcatus* to temperature gradient (Alekseev and Dubinina 2000). Genetic distinctions between borreliae infected and uninfected ticks are connected to selective pressure. The genotypes containing rare alleles of *Mdh** are infected in higher proportion (Radulovic et al. 2006).

According to Bohonak (1999), heterogeneity of water mites is accounted for their parasitism. On the other hand, hosts cause changes of the genetic status of ticks. High level of genetic divergence was revealed among ticks associated with various mussels (Edwards and Labhart 2000). The dependence was determined between rare alleles of *Mdh**, *a-Gpdh** loci and borreliae-infection of forest *I. ricinus* ticks in Slovenia (Radulovic et al. 2006). The infection of ticks along the Baikal highway with tick-borne encephalitis virus was 0.7-6.0% and with borreliae it was 20-67% (Nikitin and Antonova 2005).

Based on the analysis of polymorphic locus *a-Gpdh**, Healy (1979a, b) concluded that samples of *I. ricinus* ticks showed high heterogeneity. Jensen et al. (1999) found populations of *I. ricinus* to be heterogeneous using *Mdh** locus. Semenov et al. (2001) analyzed for the first time *Mdh* enzyme and showed heterogeneity of allopatric populations of *I. persulcatus* collected in the suburbs of St. Petersburg.

Possible reasons for the intrapopulation variability of ticks may be attributed to inadequate sampling of proteins, insufficient volume of materials and sex difference. High variability was recorded in *Amblyomma* and *Boophilus* using esterase and two isoenzymes of aconitase (Hilburn and Sattler 1986). Sexual dimorphism was detected in *I. ricinus* by *Pgi** (Bull et al. 1984) and *a-Gpdh** (Healy 1979a; Healy et al. 2004; Radulovic et al. 2006). No differences in *a-Gpdh** locus were found. It might have resulted from the insufficient number of samples (Delaye et al. 1997; Radulovic et al. 2006).

In spite of the fact that we did not carry out special studies on sex differences in allele frequencies, the balance of combined samples may be the evidence of the absence of differences between males and females. To obtain a reliable picture it is necessary to obtain information on relationship of the ecosystem components of natural infection nidi, select genetic markers and investigate large samples.

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