Ticks and Tick-borne Diseases 3 (2012) 212–218

Contents lists available at SciVerse ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.de/ttbdis

Original article

Phylogeographical structure of the tick *Ixodes persulcatus*: A novel view

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ARTICLE INFO

Article history:
Received 18 January 2012
Received in revised form 19 March 2012
Accepted 19 March 2012

Keywords:
Ixodes persulcatus
Phylogeography
Preferential amplification
rRNA
Population structure

ABSTRACT

The tick *Ixodes persulcatus* Schulze, 1930, has a wide distribution from the Baltic to the Far East and is a vector of a number of human pathogens. Thus, the study of the genetic structure and evolution of this species is of great epidemiological importance. rRNA genes were used as genetic markers to identify the phylogeographical structure of the ticks. The sequences of gene fragments of 28S (expansion segment D3) and mitochondrial 12S rRNA for 25 and 76 ticks, respectively, that had been collected in various regions of Russia in 2007–2011, were obtained. The sequences of the 28S rRNA D3 segment were identical for all ticks within the studied area. Analysis of the sequences of the mitochondrial 12S rRNA fragment revealed 4 haplotypes with one occurring at a frequency of 0.96. It is shown that the 'deep' population structure of *I. persulcatus* (McLain et al., 2001) was erroneous because of the inclusion of contaminating fungi sequences of 28S rRNA in the phylogenetic analysis. This was, possibly, due to the use of universal PCR primers that amplify the DNA of a wide range of eukaryotes, particularly of fungi which are common in samples of ticks. The influence of PCR conditions on the preferential amplification of the DNA of different organisms is also demonstrated.

Introduction

The tick *Ixodes persulcatus* Schulze, 1930, commonly known as the taiga tick, is a vector of a number of human pathogens including tick-borne encephalitis virus (Zilber, 1939), *Borrelia burgdorferi* s.l. (Kriuchechnikov et al., 1988), and *Anaplasma phagocytophilum* (Shpynov et al., 2004). This species belongs to the *Ixodes ricinus* complex – a group of ticks distributed in almost all geographic regions of the world (Xu et al., 2003). The tick *I. persulcatus* inhabits the taiga zone from the Baltic to the Russian Far East as well as Japan, northern China, Mongolia, and Kazakhstan (Fig. 1) (Filippova, 1985) and is the principal vector of tick-borne infections in Russia. Given such a wide range and epidemiological significance, the evolution of *I. persulcatus* is of great scientific interest.

To date, a lot of information concerning the morphology, biology, and ecology of *I. persulcatus* has been acquired (Alekseev et al., 2000; Filippova, 1985, 2002; Filippova and Musatov, 1996). Moreover, some attempts to study the intraspecific structure of the taiga tick based on a comparison of morphological features were made, and some differences in body size between Asian and European populations were found (Filippova and Musatov, 1996). The study by Filippova (1985) highlights the difficulty of investigating the geographic variation in this species because of the wide geographic range and the inconsistency of data on the variability of morphological structures at different developmental stages of ticks (Filippova, 1985).

Generally, morphological traits may have a complex genetic basis and are often dependent on environmental conditions. Therefore, genetic markers should be used for such population studies. They were shown to be useful in both systematic and population analyses of ticks (Nava et al., 2009). Several studies on ticks belonging to the *Ixodes ricinus* complex (Casati et al., 2008; Norris et al., 1996) allowed a comparative analysis of their genetic structure to be carried out and the processes governing tick distribution to be understood. However, little is known about the population structure of *I. persulcatus*. In this regard, special attention should be paid to the work of McLain et al. (2001) who suggested extreme heterogeneity and deep geographical structure of the *I. persulcatus* population, based on the nucleotide sequence of the expansion segment D3 28S rRNA, as well as a significant difference between the secondary structure of this segment compared to other ticks of the *I. ricinus* complex (McLain, 2001). Such a structure suggested a long and independent evolution of *I. persulcatus* populations. As pathogens transmitted by *I. persulcatus* also show heterogeneous genetic structure (Ecker et al., 1999; Fomenko et al., 2009; Nefedova et al., 2010), they can form an excellent basis for the study of coevolutionary processes in populations of pathogens and their vectors.

Since McLain et al. (2001) did not include an analysis of ticks from the central part of the range, it was decided to fill this gap and determine the genetic structure of *I. persulcatus* primarily in the Middle Urals and Western Siberia and selectively in some regions of Russia. For more accurate analysis, a fragment of the

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http://dx.doi.org/10.1016/j.ttbdis.2012.03.005

Fig. 1. The distribution range of the tick, *Ixodes persulcatus* (colored in gray). Collection sites are indicated by a black circle (●).

mitochondrial 12S rRNA gene, which has been widely used in population genetic studies of ticks (Beati and Keirans, 2001; Casati et al., 2008; Norris et al., 1996), was used as an additional marker. Our results do not support the finding of McLain et al. (2001) leading us to reconsider the existing view on the genetic structure and evolution of *I. persulcatus*.

Materials and methods

Ticks, altogether 77 (76 *I. persulcatus* and 1 *I. pavlovskyi*), were collected in various regions of Russia in 2007–2011. Sampling was carried out in such a way as to represent a large portion of the species distribution. Collection sites are indicated on the map (Fig. 1). Detailed information on the collection sites as well as GenBank accession numbers are presented in Table 1.

Extraction of nucleic acids and reverse transcription

Ticks were frozen in liquid nitrogen, crushed, and homogenized in physiological salt solution (0.154 M NaCl) in order to obtain a suspension. Nucleic acid was extracted using a Ribo-Sorb kit for RNA/DNA extraction (Interlabservis, Russia), and reverse transcription was performed using a Reverta kit (Interlabservis), both according to the manufacturer's instructions.

PCR and sequencing

The sequences of primers used for amplification are given in previously published papers (12S rRNA, Beati and Keirans, 2001; 28S rRNA, McLain et al., 2001). The PCR reaction mixture (total volume 25 μl) contained 3.0 mM $MgCl_2$, 0.4 mM dNTP, 0.4 μM of each primer, 1 U DiaTaq polymerase (Interlabservis), 5.0 μl cDNA template. PCR amplifications were performed using a Veriti™ Thermal Cycler (Applied Biosystems, USA) with the following conditions: 94 °C for 2 min; 42 cycles: melting 94 °C 10 s, annealing of primers 59 °C 10 s; synthesis of DNA 72 °C 15 s, and 72 °C 3 min. Hot start (wax barrier method) was used to improve the specificity of PCR. However, in some cases, this method was shown to be inappropriate for our purposes (see 'Results'). PCR products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide and visualized with a UV transilluminator. The DNA sequencing was performed on a Genetic Analyzer ABI PRIZM® 310 (Applied Biosystems, USA) using the reagent kit, BigDye Terminator v.3.1., according to the manufacturer's instructions.

Phylogenetic analysis

Analysis, alignment of sequences and reconstruction of phylogenetic trees were performed using MEGA 5.05 (Tamura et al., 2011). Phylogenetic trees were constructed using Neighbor-joining algorithm (all ambiguous positions were removed for each sequence pair), with bootstrapping over 500 pseudo-replicates.

Secondary structure prediction

The secondary structure of the 28S rRNA D3 segment was predicted for the sequence of *I. persulcatus* obtained in our study as well as for sequences from GenBank (*I. persulcatus* AF303997, *Cordyceps scarabaeicola* AF339524, *Mus musculus* NR_003279). The secondary structure prediction was carried out using the program, Mfold (http://mobyle.pasteur.fr/cgi-bin/portal.py?form=mfold), based on a minimum free energy algorithm (Zuker et al., 1999). The resulting structures were visualized using RnaViz (De Rijk et al., 2003).