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Sequence variability in the mitochondrial 12S rRNA and tRNA^{Val} genes of *Ixodes scapularis* (Acari: Ixodidae) individuals shown previously to be genetically invariant☆

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ABSTRACT

The DNA sequences of the mitochondrial (mt) 12S rRNA and tRNA^{Val} genes were characterized for 82 blacklegged ticks (*Ixodes scapularis*) that were genetically identical for Domains IV and V of the mt 16S rRNA gene. Thirty-one haplotypes, differed in sequence by 1–9 bp, were detected among the 82 ticks. Most nucleotide alterations in DNA sequence did not affect the stability of the secondary structures of the RNAs. The magnitude of the DNA sequence variation in the mt 12S rRNA and tRNA^{Val} genes among blacklegged ticks suggests that this region of the mitochondrial genome has potential as a genetic marker for examining the population genetics and phylogeography of *I. scapularis*.

1. Introduction

The blacklegged tick, *Ixodes scapularis*, is the vector of *Borrelia burgdorferi sensu stricto*, the causative agent of Lyme disease, and of *Anaplasma phagocytophilum*, the etiological agent of human granulocytic anaplasmosis in the Northeast and Midwest of the USA [1–4]. Prior to the 1990s, only a single population of *I. scapularis* was known to occur in Canada; on Long Point Peninsula in southern Ontario [5]. The number of resident populations of blacklegged ticks in southern Canada has increased in recent years [6]. This is associated with the introduction of *I. scapularis* larvae and nymphs into Canada from the United States by migratory passerines during their spring migration [7,8] and changing climatic conditions [9]. Determination of the geographical origins of *I. scapularis* in southern Canada is important for determining the risk of human exposure to the different species and strains of pathogens carried by blacklegged ticks.

Blacklegged ticks can be separated into two clades, the American clade and the Southern clade, based on the sequences of Domains IV and V of the mitochondrial (mt) 16S ribosomal RNA (rRNA) [10–13]. This gene has often been used as the marker in population genetic studies of *I. scapularis* [10–15] because the genome of this tick species contains very few informative microsatellite loci [16]. Individuals of the Southern clade have only been reported from North Carolina, South Carolina, Georgia, Oklahoma, Texas, Arkansas and Florida, whereas those of the American clade occur primarily in the Northeast and Midwest of the United States, but also in some southern states [10–14]. Blacklegged ticks in southern Canada also belong to the American clade [15].

Krakowetz et al. [15] reported that there were significant differences in genetic structure among established populations of *I. scapularis* in southern Canada. These authors proposed that *I. scapularis* in different geographical areas of southern Canada might be derived from colonising individuals transported from the USA by migratory passerines using different flyways [15]. To test this hypothesis, Krakowetz et al. [17] compared the DNA sequences of the mt 16S rRNA gene for *I. scapularis* from resident

☆ *Note*: Nucleotide sequences reported in this paper have been deposited in GenBank, EMBL and DDBJ databases under accession nos. HG918113–HG918174.

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populations in Manitoba and Ontario with blacklegged ticks from established populations in the Northeast and Midwest of the United States. Although different 16S haplotypes were detected among *I. scapularis* from different geographical areas, this genetic marker provided limited information regarding the potential geographical origins of *I. scapularis* populations in Canada, because a large proportion (~45%) of individuals within each population tested represented a single haplotype (i.e., haplotype Is-1) [17]. Therefore, it was concluded that additional genetic markers were needed to provide greater resolution of the phylogeographical patterns of blacklegged ticks from different regions in southern Canada [17].

Other mitochondrial genes (e.g., 12S rRNA gene and cytochrome oxidase *c* subunit 1 gene), and/or nuclear genes have been used to examine the population genetics and phylogeography of *I. scapularis* [11,18–20]. For example, Norris et al. [11] reported genetic variation among blacklegged ticks from the Northeast, Midwest and Southeast of the United States based on the DNA sequences (420 bp) of the 3′ terminal end of the mt 12S rRNA gene. However, this study was based on a small number of blacklegged ticks from both the American and Southern clades. Therefore, the objective of the our study was to determine the magnitude of genetic variability in the DNA sequences of the mt 12S rRNA gene and 3′ flanking $tRNA^{Val}$ gene among blacklegged ticks representing the 16S haplotype Is-1. Such an investigation would inform as to the usefulness of this target region as a genetic marker to study the phylogeography of *I. scapularis*.

2. Materials and methods

Eighty-two *I. scapularis* collected from established populations in southern Canada, the Northeast and Midwest of the United States, all of which were previously characterized as haplotype Is-1 (= haplotype F of Qiu et al. [12]) based on DNA sequences of Domains IV and V of the mt 16S rRNA gene [17], were used in the present study. The method used to extract the total genomic DNA of each tick has been described previously [15]. The terminal 3′ end of the mt 12S rRNA gene (~415 bp) and the flanking $tRNA^{Val}$ gene (59 bp) were amplified from genomic DNA from individual ticks by PCR using primers Tick-12S-1 (5′-AAACTAGGATTAGATACCC-3′) [11] and Iscap-16S-New-1 (5′-CATATTTAATGATATCTGGT-3′), the latter of which we designed by a comparison of the DNA sequences of the mitochondrial genomes of *Ixodes ricinus* (GenBank accession no. JN248424) and *Ixodes persculatus* (GenBank accession no. AB073725). PCRs were conducted in 25 μl volumes using a standard reaction buffer, 200 μM of each dNTP, 2.0 mM $MgCl_2$, 1 μM of each primer, 0.5 U of Fermentas recombinant *Taq* DNA Polymerase (Fisher Scientific, Canada), and 1 μl of DNA template. A no genomic DNA (i.e., negative) control was included in each set of PCRs. The cycling conditions used for the PCRs were 95 °C for 5 min, and then 35 cycles of 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min, followed by a final 5 min extension at 72 °C. Amplicons were purified by adding 1 μl of a mixture containing 3 U of exonuclease I, 0.15 U of shrimp alkaline phosphatase and 0.7 μl of PCR buffer to 10 μl of each amplicon, and incubating the samples at 37 °C for

Table 1
The number of *I. scapularis* individuals of each haplotype (HT) of the mt 12S rRNA + $tRNA^{Val}$ genes, and the variable positions in the 430 bp alignment of the DNA sequences for this region of the mitochondrial genome. A dot at an alignment position indicates the same nucleotide as in the sequence of haplotype CT08, while a dash represents a deletion.

HT	*n*	Alignment position:																											
		12S rRNA gene																								tRNA^{Val} gene			
		4 3	5 7	1 1 1	1 2 0	1 3 8	1 3 9	1 4 4	1 4 5	1 4 6	1 4 7	1 4 8	1 4 9	1 5 0	1 7 9	2 0 6	2 1 6	2 2 5	2 2 6	2 2 7	2 2 8	2 3 0	2 3 3	3 2 7	3 3 8 4	3 3 8 7	4 1 7	4 1 8
CT08	33	C	C	A	A	A	A	T	T	T	T	–	–	A	A	T	G	T	–	A	C	–	–	A	A	T	G	T
CT03	2	.	.	.	.	T	.	.	.	.	.	A	T	.	.	.	.	.	–	.	A	A	–	.	.	C	.	.
CT05	3	.	.	.	.	.	.	.	.	.	–	–	A	.	.	.	.	.	–	.	.	–	–	.	.	C	.	.
CT06	5	.	.	.	.	.	.	.	.	.	.	T	–	–	.	.	.	.	–	.	.	–	–	.	.	C	.	.
CT07	1	.	.	.	.	.	.	.	.	.	.	T	–	–	.	.	.	–	–	.	.	–	A	.	.	.	.	.
CT09	1	.	T	.	.	.	.	.	.	.	–	–	A	.	.	.	.	.	–	.	.	–	–	.	.	.	.	.
CT10	2	.	.	.	.	.	.	.	.	.	.	–	–	.	.	C	.	.	–	.	.	–	–	.	.	C	.	.
CT15	1	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	–	.	.	–	–	.	C	.	.	.
CT19	1	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	–	.	.	–	–	.	.	.	A	.
CT20	2	.	.	.	.	.	.	.	.	.	.	–	–	.	T	.	.	.	T	.	.	–	–	.	.	.	.	.
CT21	1	.	.	.	.	.	.	.	.	.	.	–	A	.	.	.	.	.	–	.	.	–	–	.	.	C	.	.
CT25	7	.	.	.	.	.	.	.	C	.	.	–	–	.	.	.	.	.	–	.	.	–	–	.	.	C	.	.
CT27	1	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	T	T	.	–	–	.	C	.	.	.
CT30	3	.	.	.	.	.	.	.	C	.	.	–	–	.	.	.	.	.	–	.	.	–	–	.	.	.	.	.
CT32	1	.	.	.	.	.	.	.	.	.	.	–	A	.	.	.	.	.	–	.	.	–	–	.	.	.	.	.
CT33	1	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	–	.	T	–	–	.	.	.	.	.
CT44	1	.	.	.	.	.	.	.	.	.	–	–	A	.	.	.	.	.	–	.	.	–	–	.	.	.	.	.
CT45	1	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	–	A	.	.	A	–	.	.	C	.	.
CT47	1	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	T	.	–	.	.	–	–	.	.	C	.	.
CT49	1	.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	–	G	.	–	–	.	.	.	.	.
CT50	1	.	.	.	.	.	.	.	C	.	.	–	–	.	.	.	.	.	–	.	.	–	–	G	.	C	.	.
CT52	1	.	.	.	.	.	.	.	C	.	–	A	T	.	.	.	.	.	–	.	.	–	–	.	.	C	.	.
CT53	1	.	.	.	.	.	.	C	.	.	.	–	A	.	.	.	.	.	–	.	.	–	–	.	.	.	.	.
CT54	1	.	.	.	.	.	G	.	.	.	.	T	T	–	.	.	.	.	–	T	T	–	–	.	.	C	.	.
CT55	1	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	.	–	.	.	–	–	.	.	.	.	.
CT56	2	.	.	.	.	.	.	.	.	.	.	T	–	.	.	.	.	.	–	.	.	–	–	.	.	.	.	.
CT57	1	.	.	.	.	.	.	.	.	A	.	–	–	.	.	.	.	.	–	.	.	–	–	.	.	.	.	C
CT58	1	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.	.	.	–	.	.	–	–	.	.	.	.	.
CT59	1	.	.	.	.	.	.	.	C	.	.	–	–	.	.	.	.	.	–	G	.	–	–	.	.	C	.	.
CT61	2	.	.	.	.	.	G	.	.	.	.	T	T	–	.	.	.	.	–	.	.	–	–	.	.	C	.	.
CT62	1	G	.	T	T	.	.	.	.	.	.	T	T	.	.	.	.	.	–	.	.	–	–	.	.	.	.	.
TOTAL	82																												