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Assessment of intraspecific mtDNA variability of European *Ixodes ricinus* sensu stricto (Acari: Ixodidae)

S. Casati [a], M.V. Bernasconi [b], L. Gern [c], J.-C. Piffaretti [a,*]

[a] *Istituto Cantonale di Microbiologia, Via Mirasole 22A, 6500 Bellinzona, Switzerland*
[b] *Zoologisches Museum, Universität Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland*
[c] *Institut de Zoologie, Laboratoire de Parasitologie, Université de Neuchâtel, 2000 Neuchâtel, Switzerland*

Abstract

The *Ixodes ricinus* complex is composed of 14 species distributed worldwide. Some members of this complex are involved in the transmission of a number of diseases to animals and humans, in particular Lyme borreliosis, tick-borne encephalitis, ehrlichiosis and babesiosis. While the phylogenetic relationships between species of the *I. ricinus* complex have been investigated in the past, still little is known about the genetic structure within the species *I. ricinus* sensu stricto. We have investigated the intraspecific variability among 26 *I. ricinus* s.s. ticks collected in various European countries, including Switzerland, Italy, Austria, Denmark, Sweden, and Finland by using five mitochondrial gene fragments corresponding to the control region, 12S rDNA, cytb, COI, and COII. The five genes considered here showed a low genetic variability (1.6–5%). Our results based on both statistical parsimony (applied to the COI + COII + cytb + 12S + CR data set, for a total of 3423 bp) and maximum parsimony (applied to the COI + COII + cytb + 12S data set, for a total of 2980 bp) did not provide any evidence for a correlation between the identified haplotypes and their geographic origin. Thus, the European *I. ricinus* s.s. ticks do not seem to show any phylogeography structure.

Keywords: Mitochondrial DNA; Control region; COI; COII; Cytb; 12S rDNA; Europe; *Ixodes ricinus*; DNA sequences; Phylogenetic analysis

1. Introduction

Ticks are obligate hematophagous arthropods parasitizing all vertebrate classes. They are currently considered to be second only to mosquitoes as vectors of human infectious diseases (Parola and Raoult, 2001). The *Ixodes ricinus* complex forms a group composed of 14 species distributed in different geographic regions of the world. Despite this geographical delocalisation, it includes ticks which are closely related (Acari: Ixodidae) (Xu et al., 2003). A number of organisms of this complex is involved in the transmission of various diseases to animals and humans such as Lyme borreliosis, tick-borne encephalitis, ehrlichiosis, and babesiosis.

In Europe, the species *I. ricinus* sensu stricto plays a central role as a vector of the agents of the above-mentioned pathologies. *I. ricinus* ticks may feed on several host species, particularly birds and mammals, including humans.

Whereas phylogenetic relationships between the members of the *I. ricinus* complex have been analysed in the past (Caporale et al., 1995; Xu et al., 2003), in contrast little is known about the genetic structure of the species *I. ricinus* s.s.. This kind of information is potentially important to understand the epidemiology and evolutionary dynamics of the disease and the vector.

Mitochondrial DNA (mtDNA) is particularly well-suited as a genetic marker for phylogenetic studies because mitochondrial genes evolve more rapidly than nuclear genes (Li, 1997). In addition, being transmitted only by the mother (i.e. the ovule), they do not undergo meiotic recombination (Simon et al., 1994). A few studies have demonstrated that their sequences are useful to distinguish closely related tick species and are thus appropriate for distinguishing populations within a species (Caporale et al., 1995; Xu et al., 2003). This is particularly the case with the control region, which appears to be subject to very weak selective constraints and evolves quite

* Corresponding author. Present address: Interlifescience, Via San Gottardo 92, 6900 Massagno, Switzerland. Tel.: +41 91 960 05 55; fax: +41 91 960 05 56.
E-mail address: piffaretti@interlifescience.ch (J.C. Piffaretti).

rapidly (Li, 1997). Its variability has made this DNA region successful for relationship studies below the species level both in vertebrates and insects (Taylor et al., 1993; Simon et al., 1994). Other mitochondrial genes such as the cytochrome oxidase subunit I (COI), the cytochrome oxidase subunit II (COII), the cytochrome b (cytb) and the 12S ribosomal RNA (12S rDNA) show appropriate characteristics, which also make them suitable for evolutionary analyses (Simon et al., 1994; Lunt et al., 1996; Caterino et al., 2000; Simmons and Weller, 2001; Bernasconi et al., 2002).

The goal of this study was to investigate the intraspecific variability at the mtDNA level of *I. ricinus* s.s. ticks originating from different European regions. For that, we used sequences from the control region, 12S, cytb, COI, and COII.

2. Materials and methods

2.1. Sample source

The 26 *I. ricinus* ticks used in this study were collected from field sites and from different hosts in various regions of Europe (Switzerland, Italy, Austria, Finland, Denmark, and Sweden) (Table 1). Ticks were stored in 100% ethanol and conserved at 4 °C until taxonomical identification (based on morphology characters) and DNA extraction. *I. hexagonus* (GenBank accession number AF081828) and *Rhipicephalus sanguineus* (GenBank accession number AF081829) sequences were used for outgroup comparison.

2.2. DNA extraction

DNA was extracted from ticks by using a Dneasy Tissue kit (Qiagen AG, Basel, Switzerland) according to the manufacturer's instructions. Each sample was cut with a disposable sterile scalpel within a 1.5 ml microtube. After digestion with proteinase K (20 μg/ml) samples were applied to the columns for DNA absorption and washing. Finally, the DNA was eluted in 200 μl of buffer available from the kit and stored at 4 °C.

2.3. PCR

PCR reactions were performed with 5 μl of the extracted DNA as template, 0,5 μM of each primer (Microsynth, Balgach, Switzerland), 1 Unit Taq Polymerase (Qiagen AG, Basel, Switzerland) in a total volume of 50 μl (buffer provided by Qiagen AG). The reaction mixtures were subjected to 10 min DNA denaturation at 94 °C, 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and elongation at 72 °C for 2 min. The reaction was completed by a further 5 min step at 72 °C. The PCR assays were performed in a DNA Thermal Cycler (PerkinElmer Applied Biosystems, Rotkreuz, Switzerland). We designed primers for the control region, cytb and COI. In addition, we modified a number of primers described in Simon et al. (1994) for the cytb, COI and COII regions (Simon et al., 1994). Amplification and sequencing primers are reported in Table 2, Fig. 3A and B.

Table 1
I. ricinus s.s. analysed

Sample code number	Geographic origin	Host	Stage	GenBank accession no.				
				Control region	12S rDNA	Cytb	COI	COII
98	Switzerland (Tegna)	Dog	F	AY945473	AY945499	AY945395	AY945447	AY945421
118	Switzerland (Castel S. Pietro)	Dog	F	AY945448	AY945474	AY945370	AY945422	AY945396
131	Sweden (Stockholm)	Dog	F	AY945453	AY945479	AY945375	AY945427	AY945401
142	Switzerland (Unrerbeinnil)	Goat	F	AY945454	AY945480	AY945376	AY945428	AY945402
143	Switzerland (Unterkulm)	Dog	F	AY945455	AY945481	AY945377	AY945429	AY945403
174	Switzerland (Zurigo)	Vegetation	F	AY945456	AY945482	AY945378	AY945430	AY945404
181	Switzerland (Villeneuve)	Bird (owl)	F	AY945457	AY945483	AY945379	AY945431	AY945405
195	Switzerland (Polleggio)	Dog	F	AY945458	AY945484	AY945380	AY945432	AY945406
277	Switzerland (Neuchâtel)	Vegetation	F	AY945459	AY945485	AY945381	AY945433	AY945407
280	Switzerland (Neuchâtel)	Vegetation	F	AY945460	AY945486	AY945382	AY945434	AY945408
636	Finland (Turku)	Dog	F	AY945461	AY945487	AY945383	AY945435	AY945409
637	Finland (Turku)	Dog	F	AY945462	AY945488	AY945384	AY945436	AY945410
638	Finland (Helsinki)	Vegetation	F	AY945463	AY945489	AY945385	AY945437	AY945411
640	Denmark (Teglstrup Hegn)	Vegetation	F	AY945464	AY945490	AY945386	AY945438	AY945412
642	Sweden (Smaland)	Vegetation	F	AY945465	AY945491	AY945387	AY945439	AY945413
644	Denmark Aebel	Vegetation	F	AY945466	AY945492	AY945388	AY945440	AY945414
645	Denmark Sejer	Vegetation	F	AY945467	AY945493	AY945389	AY945441	AY945415
646	Finland (Helsinki)	Vegetation	F	AY945468	AY945494	AY945390	AY945442	AY945416
648	Finland (Helsinki)	Vegetation	F	AY945469	AY945495	AY945391	AY945443	AY945417
683	Switzerland (Sion)	Vegetation	F	AY945470	AY945496	AY945392	AY945444	AY945418
862	Switzerland (Val du Trient)	Vegetation	F	AY945471	AY945497	AY945393	AY945445	AY945419
966	Switzerland (Finger)	Vegetation	F	AY945472	AY945498	AY945394	AY945446	AY945420
1239	Austria (Hammerfeld)	Vegetation	N	AY945449	AY945475	AY945371	AY945423	AY945397
1240	Austria (Höll)	Vegetation	N	AY945450	AY945476	AY945372	AY945424	AY945398
1244	Italy (Genova)	Dog	M	AY945451	AY945477	AY945373	AY945425	AY945399
1245	Italy (Genova)	Dog	F	AY945452	AY945478	AY945374	AY945426	AY945400

F: adult female; M: adult male; N: nymph.

Table 2
Primers used in this study for amplification (PCR) and sequencing (Seq)

mtDNA gene	Primer	Use	Strand	Sequence	Reference
Control region	CR-IR-F	PCR/Seq	Major	5′-cgg ctg gca caa gtt tcg-3′	This paper
	CR-IR-R	PCR/Seq	Minor	5′-gat aat cct tta ctc agg cat-3′	This paper
cytb	CB-J-10933	PCR/Seq	Major	5′-tat gtt tta cca tga ggt caa ata tc-3′	(Simon et al., 1994) (modified)
	CB-N-11270	PCR/Seq	Minor	5′-gct ata tta aag ttt tct gca tca-3′	This paper
COI[a]	C1-J-1484	PCR/Seq	Major	5′-tga gcc att tta ccg cga tg-3′	This paper
	C1-J-1903	Seq	Major	5′-agc ttc cgt tga cat agc-3′	This paper
	C1-J-2183	PCR/Seq	Major	5′-caa cat tta ttt tga ttt ttt gg-3′	(Simon et al., 1994)
	C1-N-2309	Seq	Major	5′-tcc aat tgc taa tat agc-′3	This paper
	C1-J-2561	Seq	Major	5′-gcc aat tcg tca att gat att-3′	This paper
	C1-J-2678	Seq	Major	5′-cct tta ttt ttt gga ata aat-3′	This paper
	C1-N-2678	PCR/Seq	Minor	5′-att tat tcc aaa aaa taa agg-3′	This paper
	C1-J-2797	PCR/Seq	Major	5′-cca cga cga tac tca gat tat c-3′	(Simon et al., 1994) (modified)
COII[a]	C2-N-3389	PCR/Seq	Minor	5′-tca taa gat cay tat cat tg-3′	(Simon et al., 1994)
	C2-J-3571	PCR/Seq	Major	5′-aga tgt aat tca ctc atg a-3′	(Simon et al., 1994) (modified)
	C2-N-3661	PCR/Seq	Minor	5′-cca caa att tct gaa cat tga cca-3′	(Simon et al., 1994)
	TK-N-3785	PCR/Seq	Minor	5′-gtt taa gag acc att gct ta-3′	(Simon et al., 1994) (modified)
	TD-N-3862	PCR/Seq	Minor	5′-aga atg aca ttc tct tgt tat-3′	(Simon et al., 1994) (modified)
12S rDNA	T1B	PCR	Minor	5′-aaa cta gga tta gat acc ct-3′	(Beati and Keirans, 2001)
	T2A	PCR	Major	5′-aat gag agc gac ggg cga tgt-3′	(Beati and Keirans, 2001)
	RipSeqJ1	Seq	Major	5′-caa aaa att atg gcg g-3′	(Bernasconi et al., 2002)
	RipSeqN1	Seq	Minor	5′-gta cat att tta gag ct-3′	(Bernasconi et al., 2002)

[a] Note: the following primer combinations were used for the COI and COII PCRs: C1-J-1484/C1-N-2678, C1-J-2797/C2-N-3361, C1-J-2183/ C2-N-3389, C1-J-2561/C2-N-3389, C1-J-2561/C2-N-3661, C2-J-3571/TK-N-3785, and C2-J-3571/TD-N-3862.

2.4. DNA sequencing

PCR products were purified using an Amicon Microcon Millipore Kit (Millipore, Milano, Italy), eluted in 20 μl of buffer and stored at 4 °C. Cycle sequencing reactions were performed in total volumes of 15 μl using an ABI Prism Big Dye Terminator Cycle Sequencing Kit (PerkinElmer Applied Biosystems, Rotkreuz, Switzerland) on an ABI Prism 310 GENETIC Analyser (PerkinElmer Applied Biosystems), according to the manufacturer's instructions. Sequences were performed in both directions.

2.5. DNA sequence alignment and analysis

The mitochondrial sequences were handled and stored with the Lasergene program Editseq (DNAstar Inc., Madison, WI, USA) and aligned separately using Megalign (DNAstar Inc.); ForCon (Raes and Van de Peer, 1999), a software tool for the conversion of sequence alignments, was also used. The partition-homogeneity test (Farris et al., 1994) implemented in PAUP*4.0b10 (Swofford, 2002) was used to test whether the different datasets (COI, COII, cytb, 12S, and CR) could be combined.

2.5.1. Phylogenetic analysis

Preliminary phylogenetic analyses were first performed with MEGA and PAUP*4.0b10 on each mitochondrial gene separately by using both Neighbour-Joining (NJ) and maximum parsimony (MP) tree reconstruction methods.

Phylogenetic analysis of the COI + COII + cytb + 12S data set was carried out with PAUP*4.0b10 using the MP method (heuristic search with stepwise addition option, TBR – tree bisection reconnection – branch swapping, and 100 additional replicates). The reliability of internal branches was assessed by bootstrapping with 1000 pseudo-replicates. Gaps were coded as missing data.

2.5.2. Populations genetics analysis

Intraspecific phylogenetic relationships among *I. ricinus* mtDNA haplotypes was inferred by a statistical parsimony method (Templeton et al., 1992) using all the five genes concatenated (COI + COII + cytb + 12S + CR) and considering gaps as 5th character state. The haplotype network was constructed using the software TCS 1.13 (Clement et al., 2000). Statistical parsimony is more convenient when investigating closely related haplotypes with a low number of mutational differences than the traditional methods of phylogeny reconstruction, which require greater number of variations. The program TCS provides the 95% parsimoniously plausible branch connections between haplotypes.

3. Results

3.1. Sequence characteristics

For each of the five mitochondrial genes considered (control region, 12S rDNA, cytb, COI and COII), we obtained and aligned 26 sequences corresponding to the 26 individual ticks examined. These sequences have been deposited in GenBank (Table 1). Table 3 summarises the results obtained (variable sites, parsimony informative sites, base composition). Codon usage was biased by the high proportion of A + T. This was

Table 3
Description of the five mtDNA genes sequenced

mtDNA sequence	PCR primers	PCR primers position	Nucleotides	No. variables sites (%)	No. Pi sites (%)	Base composition			
						A (%)	T (%)	C (%)	G (%)
tRNA-Leu (ps)	CR-IR-F	Fig. 3A	478-482	24 (5%)	13 (2.7%)	38.4	43.6	9.4	8.6
Control region (cs)	CR-IR-R								
12S rDNA (ps)									
12S rDNA (ps)	T1B	Within the gene	309-311	8 (2.5%)	3 (1.0%)	39.4	40.3	6.8	13.5
	T2A								
Cytb (ps)	CB-J-10933	Within the gene	336	9 (2.7%)	6 (1.8%)	32.5	43.8	14.0	9.8
	CB-N-11270								
COI (ps)	C1-J-1484	Fig. 3B	1520	37 (2.4%)	13 (0.8%)	30.6	41.9	14.5	13.0
	C1-J-2183								
	C1-N-2678								
	C1-J-2797								
COII (cs)	C2-N-3389	Fig. 3B	771	12 (1.6%)	9 (1.2%)	34.6	40.1	15.5	9.8
tRNA-Lys (cs)	C2-J-3571								
tRNA-Asp (ps)	C2-N-3661								
	TK-N-3785								
	TD-N-3862								

Pi sites: parsimony informative sites; ps: partial sequence; cs: complete sequence.

particularly true for the cytb and COII genes in the first position, where the A + T value is 89.5%, and for the COI gene in the second position with an A + T value of 92% (data not shown).

When considering the five genes concatenated (COI + COII + cytb + 12S + CR), each tick sample examined here had its own particular haplotype, which was different from all the other individual ticks sequenced in this study. Excluding the CR, only sample 277 (from Neuchâtel, Switzerland) and 642 (from Smaland, Sweden) shared the same haplotype for the remaining four genes (COI + COII + cytb + 12S; Fig. 1).

3.2. Phylogenetic analyses

Phylogenetic analyses were first performed on each mitochondrial gene separately. The five separated trees obtained using the NJ analysis generated similar topologies (data not shown). Because concatenation can help in the recovery of a meaningful phylogeny, we created a large dataset including the genes encoding for COI, COII, cytb, and 12S rDNA for the 26 *I. ricinus* samples and the two outgroup taxa (*I. hexagonus* and *R. sanguineus)*. Combining the data in this way was also supported by the congruence among loci, tested with the partition homogeneity test ($p = 0.15$). The CR sequences were excluded from this analysis because of the difficulties in the alignment procedure owing to the high diversity between the sequences belonging to the three different tick species considered here. The analysis on the combined sequences (2980 bp) showed (without considering the two outgroup taxa) 67 variable (2.2%) and 32 (1.1%) parsimony informative sites. The MP analysis of all the 2980 positions, with all the characters weighted equally, resulted in 78 trees of the same length (tree length = 1226; consistency index = 0.963; retention index = 0.856; rescaled consistency index = 0.825; homoplasy index = 0.037) and consequently various nodes in the MP strict consensus tree proved unresolved or with insufficient bootstrap support (Fig. 1).

3.3. Populations genetics analysis

Statistical parsimony analysis was performed using TCS on the dataset composed by all the five genes sequenced here (COI + COII + cytb + 12S + CR). Also in this case, the partition homogeneity test ($p = 0.64$) justified the combination of the five data partitions. The resulting haplotype network is shown in Fig. 2. Most of the haplotypes differ for single point mutations only. Apparently, there is no correlation between haplotypes and geographic origin of the samples.

4. Discussion

The five mitochondrial genes we have used (control region, cytb, 12S rDNA, COI and COII) are generally appropriate as

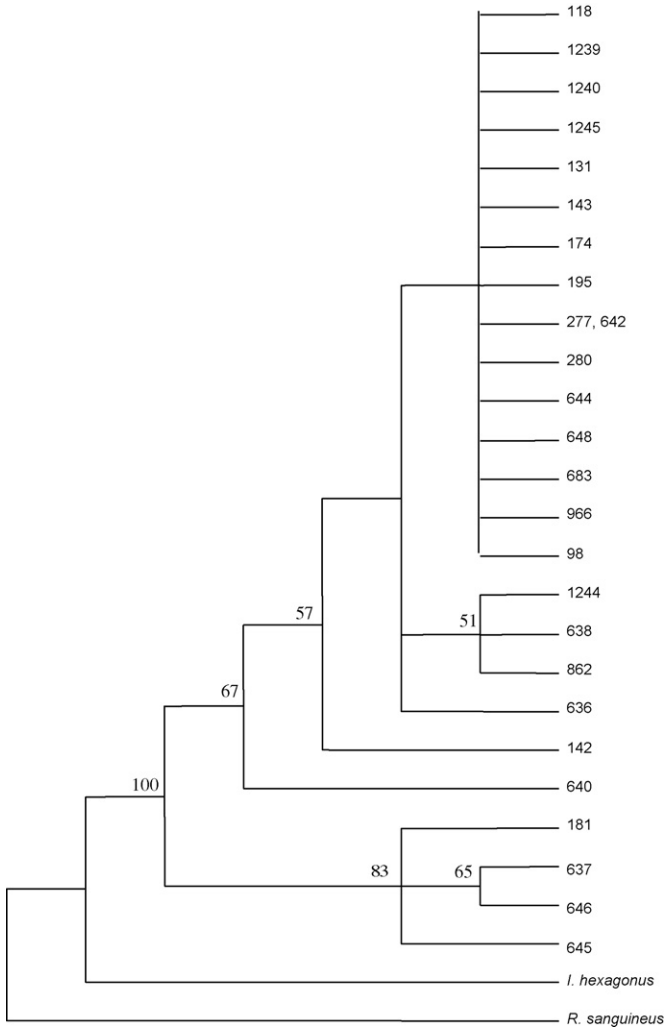

Fig. 1. MP strict consensus tree of 78 trees of the same length (tree length = 1226; consistency index = 0.963; retention index = 0.856; rescaled consistency index = 0.825; homoplasy index = 0.037) for the combined (COI, COII, cytb, 12S) data set. Bootstrap supports from 10'000 pseudo-replicates over 50% are indicated above branches. Only sample 277 (from Neuchâtel, Switzerland) and 642 (from Smaland, Sweden) shared the same haplotype for the four genes considered.

molecular markers for phylogenetic and/or population genetic studies in arthropods (Lunt et al., 1996; Bernasconi et al., 2000, 2001, 2007; Caterino et al., 2000; Simmons and Weller, 2001; Kutty et al., 2007). In our study, the number of nucleotide substitutions within the *I. ricinus* s.s. strains examined is low in all the five mitochondrial markers analysed (5.0%, 2.7%, 2.6%, 2.4%, and 1.6%, for the control region, cytb, 12S rDNA, COI, and COII, respectively). In particular, the differences among the various mtDNA haplotypes identified here originated usually from the presence of a few single point mutations. As a consequence, the parsimony informative sites are rather rare, ranging from 0.8% to 2.7%. This situation is evident in both Figs. 1 and 2, where most of the nodes resulted unresolved (Fig. 1) and most of the samples were grouped in a "bush" (Fig. 2). Thus, our data did not provide any evidence of a correlation between the identified haplotypes and their geographic origin. In other words, based on these data, the

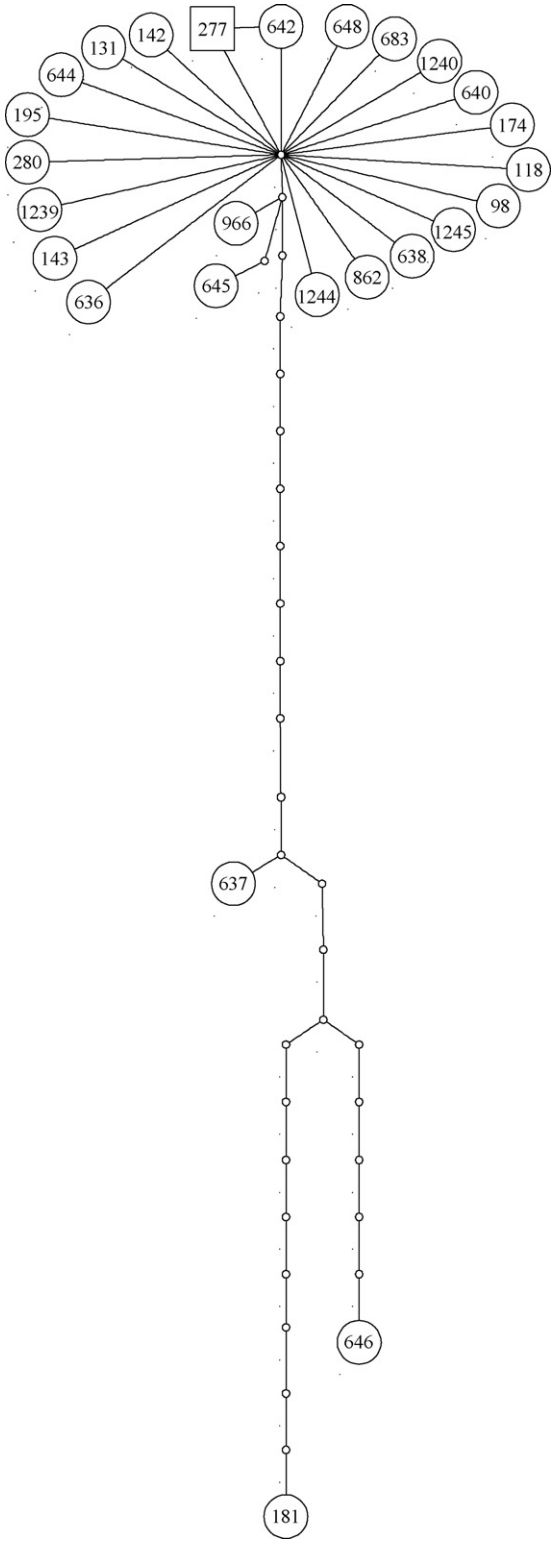

Fig. 2. Parsimony networks obtained using TCS (Clement et al., 2000), based on COI + COII + cytb + 12S + CR dataset (3423 bp) of 26 haplotypes of *I. ricinus*. Small circles indicate the number of mutational changes among haplotypes. The haplotype in a square has the biggest outgroup weight.

European *I. ricinus* s.s. ticks seems not to show any phylogeography structure.

Other studies based on allozyme data (Delaye et al., 1997) and microsatellite markers (De Meeus et al., 2002) have

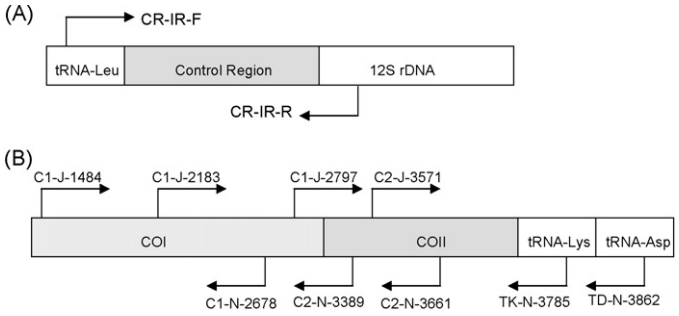

Fig. 3. PCR primers position: (A) control region: the forward primer is situated in the tRNA-Leu region, while the reverse primer is in the 12S rDNA; (B) the primers (forward and reverse) are distributed along the four regions: COI, COII, tRNA-Lys and tRNA-Asp.

provided similar results. Delaye et al. (1997) found a low variability in allozymes, which did not allow reliable population genetic conclusions in *I. ricinus* s.s. collected in the northwest part of Switzerland. De Meeus et al. (2002) found no genetic differentiation within *I. ricinus* s.s. samples originating from different regions of Switzerland separated by the Alps.

Two factors might explain the *I. ricinus* s.s. homogeneity: the first one is the recent evolution of this species (Xu et al., 2003) and the second is the host vagility (the ability to move from place to place) throughout the country.

On the base of the low number of substitutions within its members, Xu et al. (2003) hypothesised that the *I. ricinus* complex is the most recently evolved group of ticks in the genus *Ixodes* (Xu et al., 2003). In addition, our results showed a high A + T content in the DNA fragments examined. In general, it is admitted that a high A + T content is characteristic of recent evolution (Simon et al., 1994).

Recent analysis of tick-host associations does not support the generally accepted view that tick evolution arose through host adaptation, host specificity or co-speciation, but rather suggests an important role for biogeography and environment properties (Klompen et al., 1996). Indeed, despite their discontinuous geographic distribution, the habitat conditions of ticks remain very strict and are dependent on environmental conditions such as relative humidity, temperature and vegetation. Thus, *I. ricinus* s.s. ticks live in a stringent but similar environment, even though they can colonise more than 300 different avian and mammalian wild species (Gern and Humair, 2002). In addition, host movements or migrations, especially of birds (highly mobile often over large distances), may promote the uniformity among the European *I. ricinus* s.s. populations.

The suggested lack of phylogeography structure observed in Europe within *I. ricinus* s.s. ticks contrasts with the geographic genetic differentiation recorded in North America for other tick species of the *I. ricinus* complex (Norris et al., 1996; Kain et al., 1999; Qiu et al., 2002). Norris et al. (1996), using mitochondrial DNA markers (12S and 16S rDNA) and the single strand conformation polymorphism assay (SSCP), analysed the phylogeography of *I. scapularis* ticks originating from north–east America (Norris et al., 1996). They were able to identify two distinct lineages, a Southern clade (limited to the South) and an American clade (distributed in all the regions considered, but mainly in the North). These results were confirmed by Qiu et al. (2002), who analysed the mitochondrial 16S rDNA with SSCP and reported that *I. scapularis* is geographically structured across the East Coast: the northern (''American clade'') and southern (''Southern clade'') populations are genetically separated and present different evolutionary and demographic histories with an effective increase of the Northern tick population size (Qiu et al., 2002). However, allozyme analysis of the mitochondrial COIII gene of another north American tick species, *I. pacificus,* suggested weak differentiation and the tree generated from these data revealed little geographic structure for the coastal ticks, but a separation of the Utah samples (an inland state) (Kain et al., 1999). To summarise, in North-America *I. scapularis* seems to have a geographic structure more marked than *I. pacificus*, while the variability of the genetic markers analysed is approximately the same, 12.6% (53/420, 12S rDNA, (Norris et al., 1996)) and 10.1% (36/355, COIII, (Kain et al., 1999)), respectively. Thus, the two American *Ixodes* species mentioned above show a more pronounced phylogeography structure and variability (especially *I. scapularis*) than that observed for *I. ricinus* s.s. in Europe. Two factors may explain this difference. On one hand, *I. ricinus* s.s. appears to be a more recent species than *I. pacificus* and *I. scapularis*. On the other hand, the Pleistocene glaciation in the North hemisphere generated vicariance (separation and subsequent isolation of portions of an original population) for the two continents. The consequence of the glaciation period is the extinction and the loss of genetic variation of the living populations in the affected regions (Hewitt, 1996). The deep structure of *I. scapularis* and the weak structure of *I. pacificus* may reflect the past presence of different refugia populations in north–east (Qiu et al., 2002) and in north–west America, precisely in Utah (Kain et al., 1999), and on the west coast, which were not affected by the ice, with the consequent independent evolution of the enclaves (Hewitt, 1996). In contrast, the recent expansion of *I. ricinus* population northward from the South appears associated with a low degree of genetic diversity and the concomitant absence of deep genetic structure (McLain et al., 2001). This might indicate the presence of a single refugium in the south during the Pleistocene glaciation.

Interestingly, the homogeneity within the *I. ricinus* s.s. population contrasts with the extensive heterogeneity of *B. burgdorferi* s.l., which is harboured by the former (Casati et al., 2004). It has been hypothesised that the genetic diversity within the *B. burgdorferi* genospecies is driven mainly by the host, in particular by the sensitivity or resistance to its complement system (Kurtenbach et al., 2002). Thus, vertebrate hosts, rather than tick vectors, are the key to Lyme borreliosis spirochetes diversity.

In conclusion, our study provided the first attempt to unravel the genetic variability at the mtDNA level and haplotype distribution of European *I. ricinus* ticks and emphasizes the need for further research involving a larger number of both samples and localities sampled.

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