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Evolution

Distribution of the *Ixodes ricinus*-like ticks of eastern North America

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ABSTRACT **We analyzed the geographic distribution of the *Ixodes ricinus*-like ticks in eastern North America by comparing the mitochondrial 16S rDNA sequences of specimens sampled directly from the field during the 1990s. Two distinct lineages are evident. The southern clade includes ticks from the southeastern and middle-eastern regions of the United States. The range of the northern clade, which appears to have been restricted to the northeastern region until the mid-1900s, now extends throughout the northeastern and middle-eastern regions. These phyletic units correspond to northern and southern taxa that have previously been assigned specific status as *Ixodes dammini* and *Ixodes scapularis*, respectively. The expanding range of *I. dammini* appears to drive the present outbreaks of zoonotic disease in eastern North America that include Lyme disease and human babesiosis.**

The distribution of the *Ixodes ricinus*-like ticks that transmit the agents of Lyme disase (*Borrelia burgdorferi*) and human babesiosis (*Babesia microti*) in the northeastern United States appears to be in a state of flux (1). In North America, this complex of species includes *Ixodes pacificus* (2) in the West, *Ixodes scapularis* (3) in the Southeast, and *Ixodes dammini* (4) in the Northeast and Upper Midwest. Herein, we refer to these ticks as western, southern, and northern deer ticks, respectively. Before the 1950s, northern deer ticks were abundant solely in certain relict sites, including the Elizabeth Islands in Massachusetts, Long Point in Ontario, and a focus in northwestern Wisconsin (5). Their southern relatives seemed to occur throughout much of the southeastern quadrant of the United States (6). Intense mainland infestations of northern deer ticks in New England began to be recorded by the early 1960s (7) and increasingly thereafter. Although the northeastern array of populations was recognized as morphologically and biologically distinct from the southern array during the 1970s (4), the distinction has been disputed in the 1990s (8). The issue attains prominence because these ticks affect public health in the northeastern and midwestern but not the southeastern United States.

A recent comparison of nuclear sequences encoding rRNA (rDNA) of these ticks concluded that northern deer ticks cannot be distinguished from southern ticks (9). Although these sequences proved to be so variable that sibling ticks could be distinguished, no north–south pattern of variability was evident. These studies relied mainly on specimens from laboratory colonies, but they included no specimens from Florida, the extreme southern part of the range of these ticks and the best documented focus of intense southern infestations (10). Mitochondrial rDNA sequences, on the other hand, readily distinguished deer tick specimens sampled from sites in Massachusetts and Florida (11). The geographic distribution of ticks bearing these mitochondrial markers, however, has not yet been analyzed.

It may be that northern deer ticks have become established in the southern United States, forming a zone of contact within the previous range of the southern ticks. Accordingly, we sought to identify the southern limit of the range of the northern deer tick by analyzing the mitochondrial 16S rDNA sequences¶ of adult ticks sampled during the 1990s from various eastern North American sites.

MATERIALS AND METHODS

Sampling. Adult ticks were sampled from an array of sites located throughout the United States by dragging from vegetation and removal from hosts (Table 1). These field collections were designed to sample a broad geographic region (see Fig. 1). Specimens were preserved in 70% ethanol, and were identified as *I. ricinus*-like by their morphological characteristics; no *Ixodes affinis* were represented in our collections (4, 12).

PCR. Each ethanol-preserved tick was rinsed in double-distilled water, dried, and homogenized with a sterile pipette tip in 100 μl of TE (10 mM Tris·HCl, pH 8.0/1 mM EDTA). Total DNA was isolated by using a standard extraction protocol (13). The target for amplification was a major portion of the 16S rDNA gene. The primers were 16Sa (5′-CGCCTGTTTATCAAAAACAT-3′) and 16Sb (5′-CTCCGGTTTGAACTCAGATC-3′) (14). Each 50-μl reaction mixture contained 5 μl of 10× Promega *Taq* buffer, 0.2 μl of each deoxynucleoside triphosphate (10 mM), 0.2 μl of Promega *Taq* DNA polymerase, 5 μl of 16Sa primer (10 μM), 5 μl of 16Sb primer (10 μM), and about 10 ng of template. Each of the 40 cycles consisted of denaturation at 93°C for 30 s, annealing at 45°C for 1 min, and extension at 72°C for 2 min. Reactions were performed in a Perkin–Elmer model 480 thermal cycler.

Purification of Amplification Products. Double-stranded amplification products were separated by electrophoresis on a 0.8% SeaPlaque agarose gel (FMC BioProducts) in 1× TAE (40 mM Tris acetate/1 mM EDTA, pH 8.0). Amplified product sizes were compared to phage φX174 replicative form DNA *Hae* III fragments separated in the gel. Gel bands containing the ≈350-bp amplification products were melted at 65°C and digested with 1.5 μl of agarase (Sigma; 5 units/μl) at 37°C for 60 min.

Sequencing. An Applied Biosystems sequencing kit was used for *Taq* DNA polymerase-mediated incorporation of dye-labeled dideoxynucleotide terminators. Thermal cycling was performed and samples were purified and loaded on a 6%

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Abbreviation: ITS, internal transcribed spacer.

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¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U14145, U14150, U14151, U14156, U14157, U26600, U26601, U26603–26605, U26607–26613, U26615–26620, and U26622–26624).

Table 1. Origins of specimens of *I. ricinus*-like deer ticks included in this analysis

Site	Year	Collector	GenBank accession no.	Specimen code	Map code
Annandale, NY	1991	S.R.T.	U14156*	NY91	1
Ansonia, CT	1993	A. Kiszewski	U26600	CT93	2
Bedminster, NJ	1992	J. Bannister	U26610	NJ92	3
Brushy Creek, TX	1994	G. Teltow	U26624	TX94	4
Chambers County, AL	1994	G. Mullen	U26541	AL94	5
Fort Bragg, NC	1994	J. Ryan and J. F. Levine	U26617	NC94	6
Gainesville, FL	1992	S.R.T.	U26601	FL92a	7
Yarmouth, MA	1992	S.R.T.	U14145*	MA92a	8
Jacksonville, NC	1993	E. LaCombe	U14157*	NC93a	9
Langley, OK	1994	A. Kocan	U26622	OK94	10
Lawrenceville, GA	1993	J. Schlater	U26604	GA93	11
Monhegan Island, ME	1992	S.M.R.	U26611	ME92a	12
Monhegan Island, ME	1992	S.M.R.	U26612	ME92b	13
Nantucket Island, MA	1992	S.R.T.	U26607	MA92b	14
Naushon Island, MA	1992	S.R.T.	U26608	MA92c	15
New River, NC	1993	E. LaCombe	U26615	NC93b	16
Noxubee, MS	1994	J. Goddard	U26613	MS93	17
Palm Beach, FL	1992	S.R.T.	U14156*	FL92b	18
Castle Rock, IL	1994	U. Kitron	U26605	IL94	19
Prosperity, SC	1993	R. Pollack	U26623	SC93	20
Rockville, MD	1994	S.R.T.	U26609	MD94	21
Royal Palm, FL	1993	S.R.T.	U26603	FL93	22
Spooner, WI	1992	S.R.T.	U26618	WI92a	23
Spooner, WI	1992	S.R.T.	U26619	WI92b	24
Spooner, WI	1993	S.R.T.	U26620	WI93	25
Wilmington, NC	1993	E. LaCombe	U26616	NC93c	26
Yolo County, CA	1992	S.R.T.	U14150*	CA92a	27
Yolo County, CA	1992	S.R.T.	U14151*	CA92b	28

*Sequence reported previously by Caporale *et al.* (11).

polyacrylamide gel in an ABI 373A DNA sequencer (Applied Biosystems), as described elsewhere (15). Sequences were analyzed by using the SEQED 675 DNA sequence editor program (Applied Biosystems) on a Macintosh Quadra 950 computer. Each sequence was verified in duplicate by analyzing both strands of the 16S rDNA gene segment.

Phylogenetic Analysis. A multiple sequence alignment was generated by using the PILEUP program of the Wisconsin Genetics Computer Group (GCG) package (16), based on the progressive pairwise alignment algorithm (17). A 60% majority rule consensus phylogram was constructed by performing 10,000 bootstrap replications of the PAUP (18) program's heuristic tree search using the nearest-neighbor interchanges branch-swapping method. The two specimens collected from California (CA92a, CA92b) were the designated outgroup taxa for this parsimony analysis.

RESULTS

To describe phyletic divergence between the deer ticks of eastern North America, we compared the mitochondrial rDNA sequences of specimens taken directly from the field. We sampled 21 sites east of the Mississippi River, ranging from Maine to Florida, as well as sites in Oklahoma and Texas (Table 1). Sequences were deposited in the GenBank data base and registered with accession numbers. On average, 338 bp of the 16S rDNA gene were sequenced from a total of 29 ticks. A total of 47 bp (13.9%) were polymorphic, as defined by either base substitutions or insertion/deletion events.

We related the mitochondrial 16S rDNA sequences of these 29 ticks to each other in a phylogeny constructed by parsimony methods. The 60% majority rule consensus tree derived from this analysis indicates that these ticks can be assigned to two distinct clades, A and B (Fig. 1). The arithmetic mean number of base differences among all interclade pairs of individuals is equivalent to 23.7 (7.0% of all sites) (Fig. 2). Within each clade, the average base difference among all pairwise comparisons of individuals were 2.4 base differences (0.7% of all sites). Some 44 base differences separate the western deer ticks (the outgroup) from the others, and an average of 16 base differences separate the ticks from clades A and B.

We then defined the geographic distribution of the two clades of ticks and sought to identify zones of contact. Thus, the 6 specimens that represented clade B derived from the southeastern and middle-eastern faunal regions of the United States, as defined by Danks (19) (Fig. 2). The 20 specimens constituting clade A came from the northeastern and middle-eastern regions, including Alabama and Mississippi. Both clades are represented in the Carolinas, suggesting that this area is a zone of contact between divergent genotypes.

DISCUSSION

A relatively ancient divergence separates the western populations of the North American *Ixodes* deer ticks from the eastern array of populations, and another apparently more recent divergence separates the northeastern from the southeastern arrays of ticks. Indeed, those in the western clade, designated *I. pacificus*, are more closely related to Eurasian *Ixodes persulcatus* than to the more *I. ricinus*-like ticks of eastern North America (11), reflecting their possible origins in Beringia. The magnitude of the divergence between *I. pacificus* and the more eastern arrays of populations is at least three times as great as that between the northeastern (clade A) versus southeastern (clade B) populations. Although mitochondrial DNA sequence divergence may not correlate with taxonomic rank, the observed degree of difference between the 16S rDNA of the deer tick clades of eastern North America is consistent with that distinguishing other invertebrate taxa that are regarded as good species (11, 20, 21).

Although our analysis of a mitochondrial sequence resolves two eastern lineages among the deer ticks of North America,

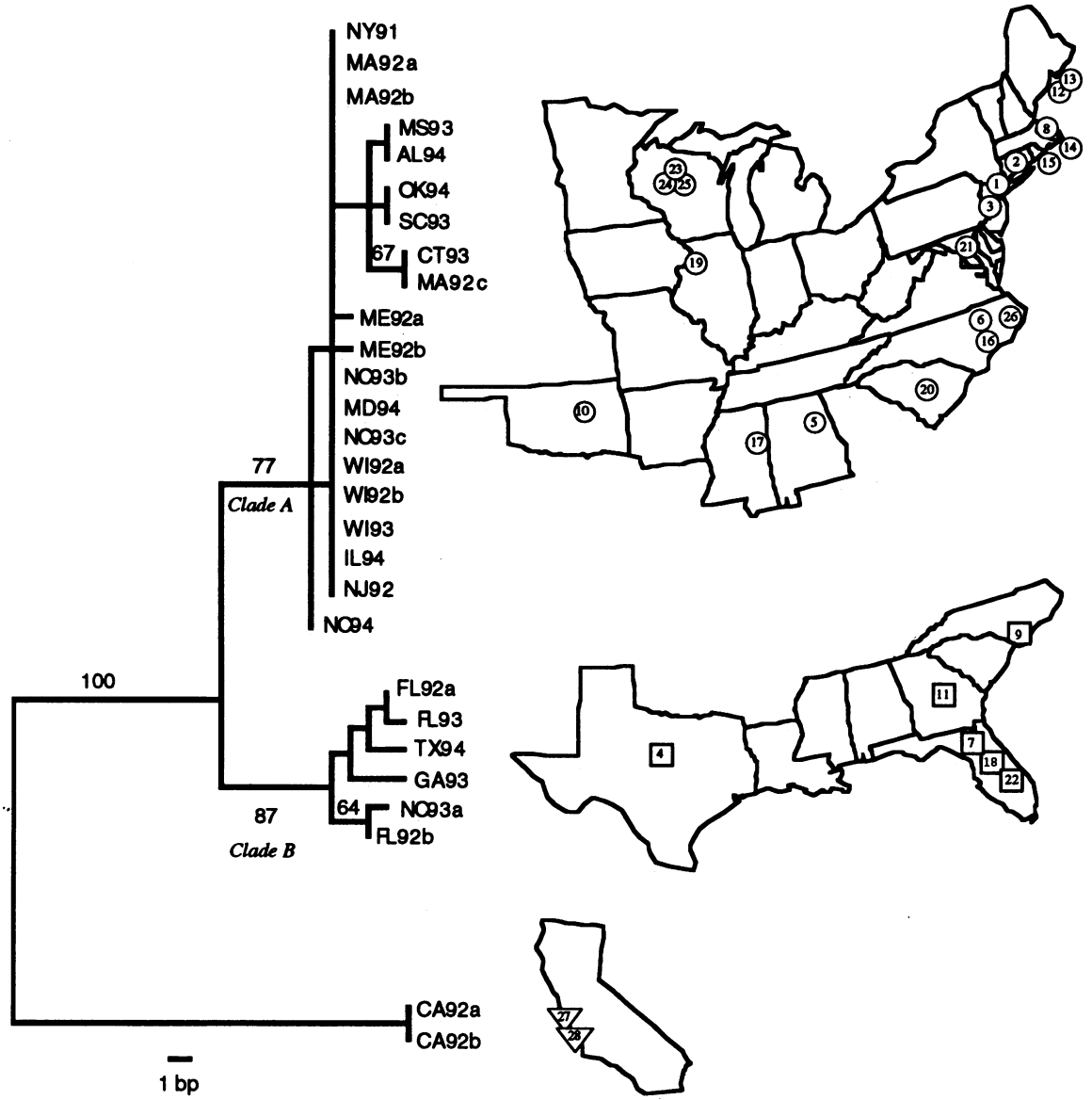


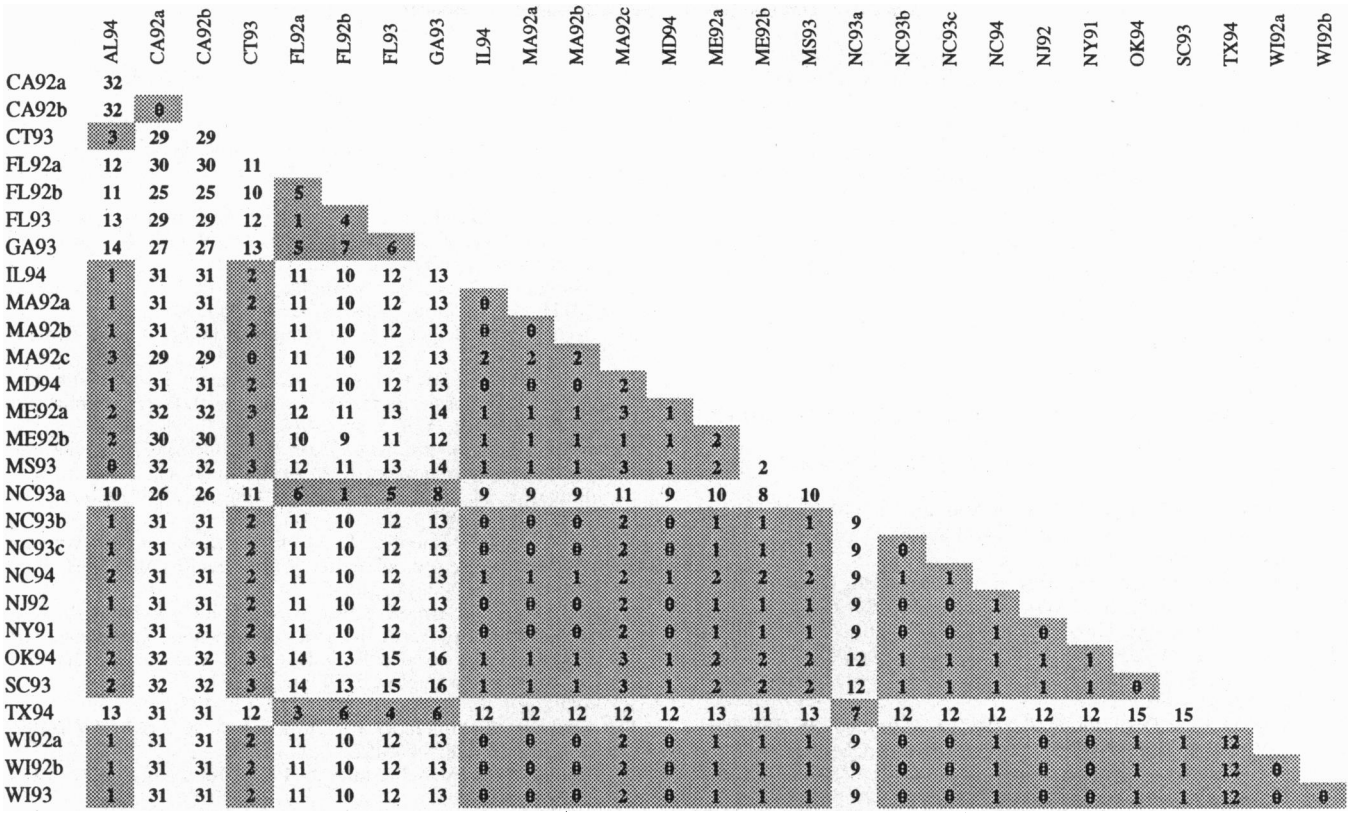
FIG. 1. Phylogeny illustrating the relationships of 28 mitochondrial 16S rDNA sequences, based on a 60% majority rule consensus of 10,000 bootstrap replications of the heuristic tree search with nearest-neighbor interchanges. Bootstrap values are provided above each major branch. Branch lengths can be inferred from the 1-bp scale at the bottom. CA92a and CA92b are the designated outgroups. The adjacent state maps indicate the distribution of the three distinct groups resolved from this phylogeny. Sites are identified in Table 1.

no such bimodality seems apparent when sequences of certain nuclear rDNAs are compared (9). Indeed, the intragenic spacer sequences of progeny from a Maryland specimen seem to be more like those of a Georgian specimen than those of their own siblings. This illogical finding probably derives from the inherent variability of the internal transcribed spacer (ITS) sequence, being members of a class of genes that tend to be hypervariable because they occur in many copies. Indeed, recombinant ITS clones from an individual mosquito (*Aedes simpsoni*) may vary by as much as 1.52% (22). Their multiple copies may become homogenized too slowly to permit resolution of recently diverged populations (23, 24). This inherent heterogeneity appears to render ITS markers noninformative for resolving such closely related populations as those of the eastern deer ticks.

Mitochondrial sequences serve more effectively as markers of lineage than do ITS nuclear markers (25) because they are maternally inherited (26), because they do not recombine, and because lineages tend to become extinct, which leads to rapid fixation of particular variants (27). The 16S mitochondrial rDNA markers, therefore, appear to provide a reliable method for distinguishing diverse populations of deer ticks.

The recent glaciers that shaped possible relict sites of the *I. ricinus*-like ticks in eastern North America (28) formed some 20,000 years before the present and retreated after about 12,000 years before the present. The degree of divergence separating the haplotypes representing the two clades is consistent with such antiquity. An average of about 5% pairwise differences occur within clade B, 2% within clade A, with 11% separating the two. If the tempo of the "molecular clock" were 2% per million years (equation 1, of ref. 29), the divergence would date back about 35,000 years. The divergence between populations within the more northerly clade A may represent 12,000 years. These heuristic projections suggest that a widespread ancestral population of deer ticks was divided by continental glaciation.

The range of the ticks that constitute the mainly northeastern clade has been dynamic, expanding southward since midcentury from various relict northern sites. A biological barrier to reinvasion of more southerly sites by ticks may have existed



	AL94	CA92a	CA92b	CT93	FL92a	FL92b	FL93	GA93	IL94	MA92a	MA92b	MA92c	MD94	ME92a	ME92b	MS93	NC93a	NC93b	NC93c	NC94	NJ92	NY91	OK94	SC93	TX94	WI92a	WI92b	WI93
CA92a	32																											
CA92b	32	0																										
CT93	3	29	29																									
FL92a	12	30	30	11																								
FL92b	11	25	25	10	5																							
FL93	13	29	29	12	1	4																						
GA93	14	27	27	13	5	7	6																					
IL94	1	31	31	2	11	10	12	13																				
MA92a	1	31	31	2	11	10	12	13	0																			
MA92b	1	31	31	2	11	10	12	13	0	0																		
MA92c	3	29	29	0	11	10	12	13	2	2	2																	
MD94	1	31	31	2	11	10	12	13	0	0	0	2																
ME92a	2	32	32	3	12	11	13	14	1	1	1	3	1															
ME92b	2	30	30	1	10	9	11	12	1	1	1	1	1	2														
MS93	0	32	32	3	12	11	13	14	1	1	1	3	1	2	2													
NC93a	10	26	26	11	6	1	5	8	9	9	9	11	9	10	8	10												
NC93b	1	31	31	2	11	10	12	13	0	0	0	2	0	1	1	1	9											
NC93c	1	31	31	2	11	10	12	13	0	0	0	2	0	1	1	1	9	0										
NC94	2	31	31	2	11	10	12	13	1	1	1	2	1	2	2	2	9	1	1									
NJ92	1	31	31	2	11	10	12	13	0	0	0	2	0	1	1	1	9	0	0	1								
NY91	1	31	31	2	11	10	12	13	0	0	0	2	0	1	1	1	9	0	0	1	0							
OK94	2	32	32	3	14	13	15	16	1	1	1	3	1	2	2	2	12	1	1	1	1	1						
SC93	2	32	32	3	14	13	15	16	1	1	1	3	1	2	2	2	12	1	1	1	1	1	0					
TX94	13	31	31	12	3	6	4	6	12	12	12	12	12	13	11	13	7	12	12	12	12	12	15	15				
WI92a	1	31	31	2	11	10	12	13	0	0	0	2	0	1	1	1	9	0	0	1	0	0	1	1	12			
WI92b	1	31	31	2	11	10	12	13	0	0	0	2	0	1	1	1	9	0	0	1	0	0	1	1	12	0		
WI93	1	31	31	2	11	10	12	13	0	0	0	2	0	1	1	1	9	0	0	1	0	0	1	1	12	0	0	

FIG. 2. Number of pairwise nucleotide differences in the 16S rDNA sequence among 24 tick populations. Shaded areas indicate comparisons between individuals within each of the three major clades of North American deer ticks: western, northeastern, and southwestern.

during the postglacial Holocene: the precolonial presence of elk in the middle Atlantic states (30) indicates that the distribution of deer was discontinuous until recently due to the effects of a meningeal worm of deer (31). Deer have recently proliferated and are now continuously distributed throughout the eastern United States. Migrating birds would carry ticks southward more effectively than northward (1, 5), placing them in sites that had previously been characterized by sparse deer herds. In this manner, northern populations of deer ticks would have invaded the range of their southern relatives; both kinds of ticks now coexist in many sites, either sympatrically or as a mosaic. Secondary introgression may take place in zones of contact. The space that once separated these populations into remote northern and southern arrays no longer exists.

The observed north–south molecular differentiation of the deer ticks of the eastern United States is consistent with their host relationships. The range of the white-footed mouse (*Peromyscus leucopus*), the main host for the subadult stages of the northern tick (1), extends southward to the Carolinas. The range of the lizards that serve as the main host for the more southern ticks generally extends northward along the Atlantic coast only to that latitude. The host associations of deer ticks suggest that their ranges may correspond to the post-Wisconsin relict distributions of their vertebrate hosts (32).

Northern deer ticks differ in various ways from those in the extreme southern United States. Morphological features, particularly the degree of projection of the nymphal auriculae, readily distinguish between northern and southern deer ticks (4). Northern ticks also seem to differ behaviorally from those in the extreme South. Although imaginal ticks from both populations feed readily on human hosts in nature, only those in more northern locations feed on people during the subadult stage of the tick (33). Recent suggestions that northern deer ticks are identical to southern ticks (8, 9) rest on an analysis of only one laboratory isolate from the southern region, and this colony may not represent those ticks that exist in nature. In an analysis of mtDNA haplotypes that similarly suggests synonymy, the southernmost sample derives only from North Carolina (34), well within the range of the northern deer tick. The much greater risk of Lyme disease in the North than in the South may reflect subtle biological differences characterizing the vector populations.

Their mtDNA haplotypes divide the deer ticks of North America into three phyletic units. Those present where Lyme disease is intensely zoonotic in the northeastern and middle-eastern regions usefully correspond to the taxon that has been designated as *I. dammini*. The western *I. pacificus* and southerly *I. scapularis* taxa seem to be associated with less intense transmission. Although geographical isolation distinguishes *I. pacificus* from the other taxa, resulting in virtually complete reproductive isolation, the inferred recent divergence of the two more easterly clades may permit interfertility. Our finding that these ticks hybridize experimentally, and interestingly, with *I. ricinus* (A. Kiszewski, personal communication) suggests the possibility of introgression. How these previously disjunct clades of ticks interact within their zones of contact and whether their gene pools retain their identity remain to be determined.

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