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Detection of *Borrelia burgdorferi* Sensu Stricto *ospC* Alleles Associated with Human Lyme Borreliosis Worldwide in Non-Human-Biting Tick *Ixodes affinis* and Rodent Hosts in Southeastern United States

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Comparative analysis of *ospC* genes from 127 *Borrelia burgdorferi* sensu stricto strains collected in European and North American regions where Lyme disease is endemic and where it is not endemic revealed a close relatedness of geographically distinct populations. *ospC* alleles A, B, and L were detected on both continents in vectors and hosts, including humans. Six *ospC* alleles, A, B, L, Q, R, and V, were prevalent in Europe; 4 of them were detected in samples of human origin. Ten *ospC* alleles, A, B, D, E3, F, G, H, H3, I3, and M, were identified in the far-western United States. Four *ospC* alleles, B, G, H, and L, were abundant in the southeastern United States. Here we present the first expanded analysis of *ospC* alleles of *B. burgdorferi* strains from the southeastern United States with respect to their relatedness to strains from other North American and European localities. We demonstrate that *ospC* genotypes commonly associated with human Lyme disease in European and North American regions where the disease is endemic were detected in *B. burgdorferi* strains isolated from the non-human-biting tick *Ixodes affinis* and rodent hosts in the southeastern United States. We discovered that some *ospC* alleles previously known only from Europe are widely distributed in the southeastern United States, a finding that confirms the hypothesis of transoceanic migration of *Borrelia* species.

Establishment of *Borrelia* sp. populations in different geographic regions is determined by natural factors (1). The maintenance of spirochete species in nature depends upon the relative abundances of their reservoir hosts and vector ticks and the intensity of host-vector interactions (2). The worldwide distribution of spirochetes from the *Borrelia burgdorferi* sensu lato complex, some of which cause Lyme disease (LD), is facilitated by the long-distance dispersal of infected ticks by migrating hosts (3–5). A hypothesis for the migration route of *Borrelia* spp. between continents was proposed, and the first evidence of transoceanic dispersal of *B. burgdorferi* sensu stricto was presented almost 15 years ago (6–9).

B. burgdorferi sensu stricto is the primary, but not the only, species that causes LD around the world (10–13). Different strains of *B. burgdorferi* sensu stricto exhibit considerable genetic heterogeneity locally as well as globally. Also, molecular analyses revealed a close relationship and an overlapping of genotypes between European and North American spirochete populations, which confirms the transoceanic migration hypothesis and the existence of recombinant genotypes (6, 9). Multiple genotypes of *B. burgdorferi* sensu stricto have been identified based on the analysis of a spirochete gene (*ospC*) that encodes highly polymorphic outer surface protein C (14–16). *Borrelia* OspC antigen is heavily targeted by the host immune system. It establishes the secondary immune response, or immune memory, in hosts (17). Associations between *ospC* genotypes and invasiveness in patients (18–22) and experimentally infected animals (23, 24) have been reported. The *ospC* gene is more diverse than any other *Borrelia* gene studied to date (17). *B. burgdorferi* sensu stricto has the ability to infect a wide range of phylogenetically diverse vertebrate hosts, which facilitates the further expansion of the spirochete into new geographical areas (25–28). Selection pressure from the vertebrate immune system is likely responsible for the high level of polymorphism of the *ospC* gene (17, 29–31).

Furthermore, because *B. burgdorferi* sensu stricto is a host generalist that occurs in birds, rodents, and other mammals, its dispersal potential is considerable. More than 240 animal species have been reported as hosts for tick vectors and potential reservoir hosts of *Borrelia* in Europe (27). Such a diverse host spectrum may lead to the establishment of new enzootic LD foci in Europe. We believe that the current distribution of *B. burgdorferi* sensu stricto in Europe is much wider than has been reported and is enhanced by the involvement of multiple phylogenetically diverse migratory animal species. Such expansion could affect LD risk and helps to explain the observed increased incidence of LD in humans worldwide.

Previous studies carried out in areas where LD is endemic demonstrated that the vast majority of known *ospC* alleles are geographically distinct (7, 16, 32, 33). The presence of *Borrelia* spp. in nature is known to be affected by recent urbanization, an increasing overlap between human and *Borrelia* habitats, and climate change (9, 34–39). Thus, it is not unexpected that the distributions of *Borrelia* genotypes may have been shifting in recent decades and may continue to shift. The number of LD cases worldwide has increased recently (40, 41),

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which may be attributable to *Borrelia* expansion or to gene transfer that resulted in recombinant genotypes (31).

The objectives of this study were to compare *ospC* alleles from a southeastern U.S. population of *B. burgdorferi* sensu stricto with other *Borrelia* sensu stricto strains from North American and European localities where LD is endemic and where it is not endemic. The search for evidences that support the hypothesis of transoceanic migration of *Borrelia* species was another aim of our project. Our study was not meant to be a statistical analysis with emphasis on the ranking of *Borrelia ospC* alleles but rather an invitation to an open discussion to advance the natural history and understanding of the enzootiology of *B. burgdorferi* sensu stricto in the southeastern United States, previously considered to be a "low-or-no" Lyme disease region.

MATERIALS AND METHODS

Control sequences. As a control group, 100 *B. burgdorferi* sensu stricto strains with different *ospC* types were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/) (Table 1).

Experimental samples. The experiment group included 127 samples of *B. burgdorferi* sensu stricto. The sampling in the presented study was not exhaustive or random for a variety of reasons, as statistical analysis was not a goal of this project. Of these samples, 58 were derived from the vector ticks *Ixodes ricinus*, *I. affinis*, *I. pacificus*, and *I. scapularis*. For this purpose, each tick was rinsed in 10% bleach for 50 min, followed by a 5-min wash in 70% ethyl alcohol. The cleaned tick was air dried on sterile filter paper, placed into 100 μl of BSK-H medium (a modified Barbour-Stoenner-Kelly medium), and homogenized. All instruments and breakers were autoclaved prior to use. The homogenized mixture was transferred into 5 ml with BSK-H medium, and tubes were maintained at 34°C for 8 weeks. All work was conducted under a sterile biohazard hood.

Another 35 samples originated from the rodents *Peromyscus gossypinus*, *Neotoma floridana*, *Sigmodon hispidus*, *Tamias senex*, *Neotoma fuscipes*, and *Sciurus griseus*. Samples from ear clips were prepared as follows: a triangle cut from the ear was washed in 70% ethyl alcohol for 4 to 5 min. After that, the ear clip was soaked for 4 to 5 min in freshly diluted 10% bleach, followed by a 1-min rinse in 95% ethanol. After 2 min of air drying under sterile conditions, tissue was cut into 3 to 4 pieces and placed into 5 ml of BSK-H medium. Tubes were kept at 34°C for 6 weeks. Spirochete cultures from internal organs were initiated as follows: organs were removed from euthanized animals, placed directly into 200 μl of BSK-H medium, chopped in it, and left at room temperature for 2 to 3 min. After that, 100 μl of the mixture was transferred into 5 ml of BSK-H medium and kept at 34°C for 6 weeks. The remaining 34 samples were of human origin. Fifty-three samples were collected in Georgia, South Carolina, and Florida in the southeastern United States; 25 samples were collected in California in the far-western United States; and 49 samples came from a subset of European countries where LD is endemic, i.e., the Czech Republic, Germany, Hungary, Slovakia, Slovenia, and Switzerland (Table 2).

DNA purification, PCR amplification, and sequencing. Total *Borrelia* DNA was purified by using the DNeasy blood and tissue kit (Qiagen). Partial *ospC* genes were amplified by using previously described *ospC* primers (16) and protocols (38, 42, 43). PCR products from the European and southeastern U.S. samples were sequenced at the University of Washington, while *ospC* products from California strains were sequenced at the University of California, Berkeley, sequencing facility. All 127 samples were sequenced at the *ospC* locus directly in both directions and then assembled and edited by using DNAStar (DNAStar, United Kingdom). The BLASTN algorithm was used to confirm identity against GenBank data.

Data analysis. Sequences were aligned by using Clustal X (44). Because a high level of recombination was confirmed for the *Borrelia ospC* gene, a cladistic analysis was deemed inappropriate (45). Clustering analysis was performed by using the neighbor-joining method with uncorrected (raw) pairwise sequence distances, as modified in BioNJ (46). One thousand bootstrap replicates were performed under a neighbor-joining search to obtain support values for clusters. A 50% majority-rule consensus tree was formed, with ties broken randomly if encountered.

Nucleotide sequence accession numbers. All sequences obtained in this study have been submitted to the GenBank database under accession numbers listed in Table 2.

RESULTS

The comparative analysis presented here includes partial sequences of *ospC* genes from 227 *Borrelia* strains (100 control and 127 experimental strains) originating from areas in Europe and the northeastern, midwestern, and far-western United States where LD is recognized to be endemic and from the southeastern United States, which, for a long time, was considered to be a region where LD was not endemic.

The southeastern U.S. samples of *Borrelia* contained 22 strains isolated from *I. affinis*, 1 from *I. scapularis*, 25 from *P. gossypinus*, 1 from *N. floridana*, and 4 from *S. hispidus*. California samples included 20 isolates from *I. pacificus*, 3 from *Sciurus griseus*, 1 from *Tamias senex*, and 1 from *Neotoma fuscipes*. Among 49 European *Borrelia* samples, 15 originated from *I. ricinus*, and 34 originated from humans. Most *ospC* amplicons were 525 to 610 bp long (depending upon which PCR primers were used) and were truncated to 498 bp to achieve a perfect alignment. Four samples had to be removed from the neighbor-joining analysis because they were too short (<200 bp). These samples were placed into clusters with samples whose sequences were identical over the length of the sequence fragment that we were able to obtain for them on the assumption that this represents the best clustering position for the sequence available.

ospC sequences from the experimental samples were identified as one of the known *ospC* alleles by their strong clustering with control sequences in the analysis. In total, 14 *ospC* alleles were identified among the 127 experimental samples (Fig. 1).

Clades B, D, E3, F, G, H, H3, I3, L, M, Q, R, and V contained experimental samples and were all well supported (bootstrap values of 100%) with apparent monophyly of the clade type samples (those taken from GenBank and designated previously). Clade A received 81% bootstrap support when all samples were included; but ignoring one control sample (X84738), the clade received 100% support. In one anomaly, the *ospC* allele E3 clade, which included samples from California and one control *ospC* allele E3 strain, clustered tightly with one control type O strain from the northeastern United States. The other control *ospC* allele O strain was placed onto a separate branch, alone. This is evidence that what has been classified as *B. burgdorferi* sensu stricto genotype O is not monophyletic. Overall, clades containing experimental samples were scattered throughout the tree and represented a broad variety of *ospC* alleles.

North American samples. *ospC* allele G, the third most abundant type among experimental samples, was detected only in North American samples. It was present equally in vector- and host-derived strains from the southeastern United States that clustered with strains from the northeastern United States. *ospC* allele G was also detected in 2 *I. pacificus* nymphs.

ospC allele H was the fourth most abundant allele detected among southeastern U.S. *B. burgdorferi* sensu stricto strains cultured from ear clips, bladders, spleen, kidney, and hearts of multiple rodent hosts and a single *I. affinis* nymph. *ospC* allele H strains from California were cultured from an *I. pacificus* nymph and a western gray squirrel (*S. griseus*). This *ospC* allele was not

TABLE 1 *Borrelia burgdorferi* sensu stricto reference strains used in this study

ospC type	*B. burgdorferi* sensu stricto strain[a]	GenBank accession no.	Location	Source
A	132a	DQ437446	NE USA	Human
A	CS1	DQ437464	NE USA	*I. scapularis*
A	CS2	DQ437465	NE USA	*I. scapularis*
A	CS3	DQ437466	NE USA	*I. scapularis*
A	PKa2	EF537420	Europe	Human
A	IP1	EF537422	Europe	Human
A	OC1	AF029860	New York	Tick
A	132b	DQ437447	NE USA	Human
A	NA	EU482041	New York	Human
A	Ip2	L42887	France	Human
A	2-1498 CA4	L81131	California	Human
A	HII	U91792	Italy	Human
A	IP3	U91797	France	Human
A	L5	U91798	Austria	Human
A	IP1	U91799	France	Human
A	P1F	U91801	Austria	Human
A	PKa	X69589	Germany	Human
A	TXGW	X84783	Texas	Human
A	B31	U01894	New York	Unknown
B(nt59)	SMT44	FJ932735	California	*I. pacificus*
B1	MI415	EF537413	Michigan	*P. leucopus*
B2	ZS7	L42868	Germany	Tick
B2	Lx36	EF537411	Europe	Human
B	OC2	AF029861	New York	Tick
B	NA	EU482042	New York	Human
B	35B808	U91794	Germany	Tick
B	61BV3	U91795	Germany	Human
B	DK7	X73625	Denmark	Human
B	PBre	X81522	Germany	Human
B	BUR	X84765	New York	Human
C	OC3	AF029862	New York	Tick
C	JD1	DQ437462	NE USA	*I. scapularis*
C	NA	EU482043	New York	Human
D	OC4	AF029863	New York	Tick
D	NA	EU482044	New York	Human
D	CA-11.2A	L25413	California	Unknown
E3	HRPW89	FJ932732	California	*I. pacificus*
E	OC5	AF029864	New York	Tick
E	OC7	AF029866	New York	Tick
E	88a	DQ437459	New York	Human
E	NA	EU482045	New York	Human
E	28691	L42894	Pennsylvania	*I. scapularis*
E	N40	U04240	Connecticut	Rodent
F	27579	L42896	Connecticut	*I. scapularis*
F	*B. pacificus*	X83555	California	*I. pacificus*
F	OC6	AF029865	New York	Tick
F	NA	EU482046	New York	Human
F	2-1498 Son 188	L81130	California	Human
G	OC8	AF029867	New York	Tick
G	NA	EU482047	New York	Human
G	72a	DQ437456	New York	Human
H	OC9	AF029868	New York	Tick
H	MI411	EF537400	Michigan	*T. striatus*
H	NA	EU482048	New York	Human
H3	MCCP65	FJ932733	California	Tick (nymph)
I	OC10	AF029869	New York	Tick
I	297	L42893	Connecticut	Unknown
I	HB19	U04281	Connecticut	Human
I	NA	EU482049	New York	Human
I3	HPS6	FJ932734	California	Tick
J	OC11	AF029870	New York	Tick
J	118a	DQ437444	New York	Human
J	NA	EU482050	New York	Human
J	MIL	U91802	Slovakia	*I. ricinus*
K	OC12	AF029871	New York	Tick
K	OC13	AF029872	New York	Tick
K	28354	L42895	Maryland	*I. scapularis*
K	297	U08284	Connecticut	Human
K	MUL	X84779	New York	Human
K	KIPP	X84782	New York	Human
K	272	X84785	Connecticut	Human
K	NA	EU482051	New York	Human
L	Y1	EF537402	Europe	*I. ricinus*
L	Bol6	EF537406	Europe	*I. ricinus*
L	21347	L42899	Wisconsin	*P. leucopus*
L	T255	X81524	Germany	*I. ricinus*
M	NA	EU482052	New York	Human
M	2591	U01892	Connecticut	*P. leucopus*
N	80a	DQ437457	New York	Human
N	CS8	DQ437470	New York	*I. scapularis*
N	MI418	EF537430	Michigan	*P. leucopus*
N	NA	EU482053	New York	Human
N	26815	L42897	Connecticut	Chipmunk
O	NA	EU482056	New York	Human
O	DUNKIRK	X84778	New York	Human
P	20006	U91796	France	*I. ricinus*
Q	Bol15	EF537398	Europe	*I. ricinus*
Q	212	U91790	France	*I. ricinus*
R	Esp1	U91791	Spain	*I. ricinus*
R	NE56	U91800	Switzerland	*I. ricinus*
S	Bol26	EF537417	Europe	*I. ricinus*
S	Z136	U91793	Germany	*I. ricinus*
T	NA	EU482054	New York	Human
U	94a	DQ437460	New York	Human
U	CS5	DQ437467	New York	*I. scapularis*
U	NA	EU482055	New York	Human
V	Bol29	EF537407	Europe	Human
V	Bol30	EF537408	Europe	Human
W	Ri5	EF537414	Finland	*I. ricinus*
X	SV1	EF537427	Finland	*I. ricinus*

[a] NA, not applicable.

detected in ticks or in hosts collected from any European locality sampled in this study (Table 2).

California-specific *ospC* alleles E3 and H3 were both detected in hosts and *I. pacificus* ticks, the main vector of spirochetes from the *B. burgdorferi* sensu lato complex from the far-western United States. *ospC* alleles D, I3, and M were detected in *I. pacificus* only. The neighbor-joining analysis revealed that the only experimental samples that fell into the H3 and I3 clades were from California. Californian *ospC* alleles D and M clustered with northeastern *B. burgdorferi* sensu stricto allele D and M control sequences. *ospC* alleles D, E3, H3, I3, and M were not detected among the European or southeastern U.S. samples.

The southeastern U.S. strains of *B. burgdorferi* sensu stricto contained *ospC* alleles B, G, H, and L, with an almost equal representation of alleles B, G, and L (30% each). California samples showed the largest number of *ospC* alleles (A, B, D, E3, F, G, H, H3, I3, and M) found at any one location. *ospC* allele L was not found in California,

TABLE 2 Experiment group of the 127 *B. burgdorferi* sensu stricto samples derived from vector ticks, rodent hosts, and humans in selected European countries, the southeastern United States, and California and analyzed in this study

Region studied and *ospC* type[a]	Isolate[b]	GenBank accession no.	Location/yr of collection	Host or vector	DNA isolation source
Europe					
Q	NE5220	JQ253799	Switzerland/2010	*I. ricinus*	Spirochete culture
L	NE5222	JQ353800	Switzerland/2010	*I. ricinus*	Spirochete culture
V	NE5248	JQ352801	Switzerland/2010	*I. ricinus*	Spirochete culture
R	NE5261	JQ352802	Switzerland/2010	*I. ricinus*	Spirochete culture
B	NE5264	JQ352803	Switzerland/2010	*I. ricinus*	Spirochete culture
L	NE5266	JQ352804	Switzerland/2010	*I. ricinus*	Spirochete culture
R	NE5267	JQ352805	Switzerland/2010	*I. ricinus*	Spirochete culture
L	SKT-2	AY597021	Slovakia/2004	*I. ricinus*	Spirochete culture
B	SKT-9	AY597028	Slovakia/2004	*I. ricinus*	Spirochete culture
L	SLV 1	JQ236853	Slovenia/2006	Human	Spirochete culture
B	SLV 2	JQ236854	Slovenia/2006	Human	Spirochete culture
R*	S277(+2)	JF754968	Czech Republic/2010	*I. ricinus*	Whole tick
R	B4/N39Aug	JF754971	Germany/2010	*I. ricinus*	Whole tick
Q**	S1/11(+1)	JF754969	Czech Republic/2010	*I. ricinus*	Whole tick
V	H	JQ219681	Hungary/2000	Human	Human skin
B***	Brno35(+8)	JQ219682	Czech Republic/2009	Human	Serum/joint fluid
A	N 12	JQ219683	Czech Republic/2008	Human	Human serum
B****	N103(+20)	JQ219684	Czech Republic/2010	Human	Serum/joint fluid
USA					
B	BUL1	JF723215	GA/1994	*N. floridana*	Spirochete culture
G	BUL3	JF723216	GA/1994	*P. gossypinus*	Spirochete culture
B	BUL4	JF723217	GA/1994	*P. gossypinus*	Spirochete culture
B	BUL5	JF723218	GA/1994	*P. gossypinus*	Spirochete culture
B	BUL6	JF723219	GA/1994	*P. gossypinus*	Spirochete culture
G	BUL8	JF723220	GA/1995	*P. gossypinus*	Spirochete culture
B	BUL10	JF723221	GA/1997	*P. gossypinus*	Spirochete culture
H	MI 1	JF723262	FL/1992	*P. gossypinus*	Spirochete culture
G	SCCH 3	JF723222	SC/1995	*I. scapularis*	Spirochete culture
B	SCCH 9	JF723223	SC/1995	*P. gossypinus*	Spirochete culture
B	SCCH 13	JF723224	SC/1995	*P. gossypinus*	Spirochete culture
B	SCCH 19	JF723226	SC/1995	*P. gossypinus*	Spirochete culture
L	SCCH 24	JF723228	SC/1995	*S. hispidus*	Spirochete culture
G	SCCH 25	JF723229	SC/1995	*S. hispidus*	Spirochete culture
G	SCCH 28	JF723231	SC/1995	*S. hispidus*	Spirochete culture
L	SCCH 30	JF723232	SC/1995	*P. gossypinus*	Spirochete culture
G	SCCH 31	JF723233	SC/1995	*P. gossypinus*	Spirochete culture
G	SCGT 4	JF723263	SC/1995	*P. gossypinus*	Spirochete culture
G	SCGT 7	JF723264	SC/1995	*I. affinis*	Spirochete culture
L	SCGT 16	JF723265	SC/1995	*P. gossypinus*	Spirochete culture
H	SCGT 17	JF723266	SC/1995	*P. gossypinus*	Spirochete culture
L	SCI 1	JF723234	GA/1993	*P. gossypinus*	Spirochete culture
B	SCI 3	JF723235	GA/1993	*P. gossypinus*	Spirochete culture
G	SCSC 2	JF723267	SC/1995	*P. gossypinus*	Spirochete culture
G	SCSC 3	JF723268	SC/1995	*I. affinis*	Spirochete culture
G	SCSC 5	JF723269	SC/1995	*I. affinis*	Spirochete culture
G	SCSC 6	JF723270	SC/1995	*I. affinis*	Spirochete culture
B	SCW 1	JF723242	SC/1994	*P. gossypinus*	Spirochete culture
H	SCW 2	JF723243	SC/1994	*P. gossypinus*	Spirochete culture
B	SCW 3	JF723244	SC/1994	*P. gossypinus*	Spirochete culture
H	SCW 4	JF723245	SC/1994	*P. gossypinus*	Spirochete culture
H	SCW 6	JF723246	SC/1994	*P. gossypinus*	Spirochete culture
L	SCW 9	JF723247	SC/1994	*S. hispidus*	Spirochete culture
L	SCW 12	JF723248	SC/1994	*P. gossypinus*	Spirochete culture
B	SCW 25	JF723249	SC/1994	*P. gossypinus*	Spirochete culture
L	SCW 43	JF723250	SC/1995	*I. affinis*	Spirochete culture
L	SCW 44	JF723251	SC/1995	*I. affinis*	Spirochete culture
H	SCW 47	JF723252	SC/1995	*I. affinis*	Spirochete culture

(Continued on following page)

TABLE 2 (Continued)

Region studied and *ospC* type	Isolate	GenBank accession no.	Location/yr of collection	Host or vector	DNA isolation source
L	SCW 48	JF723253	SC/1995	*I. affinis*	Spirochete culture
B	SCW 53	JF723254	SC/1995	*I. affinis*	Spirochete culture
L	SCW 54	JF723255	SC/1995	*I. affinis*	Spirochete culture
G	SCW 57	JF723256	SC/1995	*I. affinis*	Spirochete culture
B	SCW 58	JF723257	SC/1995	*I. affinis*	Spirochete culture
G	SCW 59	JF723258	SC/1995	*I. affinis*	Spirochete culture
L	SCW 60	JF723259	SC/1995	*I. affinis*	Spirochete culture
L	SCW 61	JF723260	SC/1995	*I. affinis*	Spirochete culture
B	SCW 62	JF723261	SC/1995	*I. affinis*	Spirochete culture
L	SI 14	JF723236	GA/1995	*I. affinis*	Spirochete culture
B	SI 15	JF723237	GA/1995	*I. affinis*	Spirochete culture
L	SI 16	JF723238	GA/1995	*I. affinis*	Spirochete culture
L	SI 17	JF723239	GA/1995	*I. affinis*	Spirochete culture
G	SI 18	JF723240	GA/1995	*I. affinis*	Spirochete culture
L	SI 19	JF723241	GA/1995	*I. affinis*	Spirochete culture
D	BOR4	JQ308215	CA/2004	*I. pacificus*	Spirochete culture
H	BOR53	JQ308216	CA/2004	*I. pacificus*	Spirochete culture
E3	BTE68	JQ308217	CA/2004	*I. pacificus*	Spirochete culture
A	BTW11	JQ308218	CA/2004	*I. pacificus*	Spirochete culture
E3	BTW16	JQ308219	CA/2004	*I. pacificus*	Spirochete culture
H3	BTW37	JQ308220	CA/2004	*I. pacificus*	Spirochete culture
G	BTW52	JQ308221	CA/2004	*I. pacificus*	Spirochete culture
M	BTW62	FJ932736	CA/2004	*I. pacificus*	Spirochete culture
H3	BTW67	JQ308222	CA/2004	*I. pacificus*	Spirochete culture
D	CHRW46	JQ308223	CA/2004	*I. pacificus*	Spirochete culture
A	CHRW57	JQ308224	CA/2004	*I. pacificus*	Spirochete culture
A	FCR13	JQ308225	CA/2004	*I. pacificus*	Spirochete culture
F	HOPK32	JQ308226	CA/2004	*I. pacificus*	Spirochete culture
G	HOPN45	JQ308227	CA/2004	*I. pacificus*	Spirochete culture
I3	HPS6	FJ932734	CA/2004	*I. pacificus*	Spirochete culture
I3	HPS61	JQ308235	CA/2004	*I. pacificus*	Spirochete culture
E3	HRPW89	FJ932732	CA/2004	*I. pacificus*	Spirochete culture
B	HUMB27	JQ308233	CA/2004	*T. senex*	Spirochete culture
E3	HUMB150	JQ308234	CA/2004	*N. fuscipes*	Spirochete culture
E3	LAG24	JQ308228	CA/2004	*I. pacificus*	Spirochete culture
H3	LMSW22	JQ308229	CA/2004	*I. pacificus*	Spirochete culture
H3	MCCP65	FJ932733	CA/2004	*I. pacificus*	Spirochete culture
H3	SGE03-1	JQ308230	CA/2003	*S. griseus*	Spirochete culture
H	SGE03-4	JQ308231	CA/2003	*S. griseus*	Spirochete culture
A	SGE03-7	JQ308232	CA/2003	*S. griseus*	Spirochete culture

[a] Asterisks indicate representation by 3 (*), 2 (**), 9 (***), or 21 (****) identical sequences.
[b] Additional identical strains in group are indicated in parentheses.

despite it being widely distributed in ticks and hosts in the southeastern United States and connected to human LD in Europe.

European samples. *ospC* alleles Q, R, and V were found among the European *B. burgdorferi* sensu stricto samples only (Table 2). Clades Q and R contained only vector-originated spirochete samples from control and experimental groups; clade V contained both tick- and human-originated strains (Fig. 1).

The highest level of diversity of *B. burgdorferi* sensu stricto *ospC* alleles (type per number of samples) was detected in Neuchâtel, Switzerland. Five out of six (83%) *ospC* alleles detected among all European samples were found in seven spirochete cultures isolated from *I. ricinus* nymphs collected from this single location in Switzerland: *ospC* alleles B (one culture), L (two), Q (one), R (two), and V (one) (Table 2).

Transcontinental samples. Three *ospC* alleles (A, B, and L) were detected in European and North American *B. burgdorferi* sensu stricto samples.

ospC allele A, associated with the most pathogenic strains of *B. burgdorferi* sensu stricto, was detected in a serum sample from a patient (N12/JQ219683) with a LD diagnosis from the southern Czech Republic. Four other *ospC* allele A strains were identified among the Californian isolates (Table 2). All five *ospC* sequences were 100% identical and clustered clearly (100% bootstrap support) with control sequences from European countries where LD is endemic as well as from the northeastern and midwestern United States. *ospC* allele A was not detected in the samples from the southeastern United States (Table 2).

ospC allele B was the most abundant among the European samples of human origin (skin, blood, serum, cerebrospinal fluid, or joint fluid of the patients) and was the most represented among the host- and vector-originated southeastern U.S. strains (Table 2). One *ospC* allele B strain was cultivated from *T. senex* (Allen's chipmunk) captured in California. The *ospC* allele B clade consisted of two subclades, one containing a preponderance of southeastern U.S.

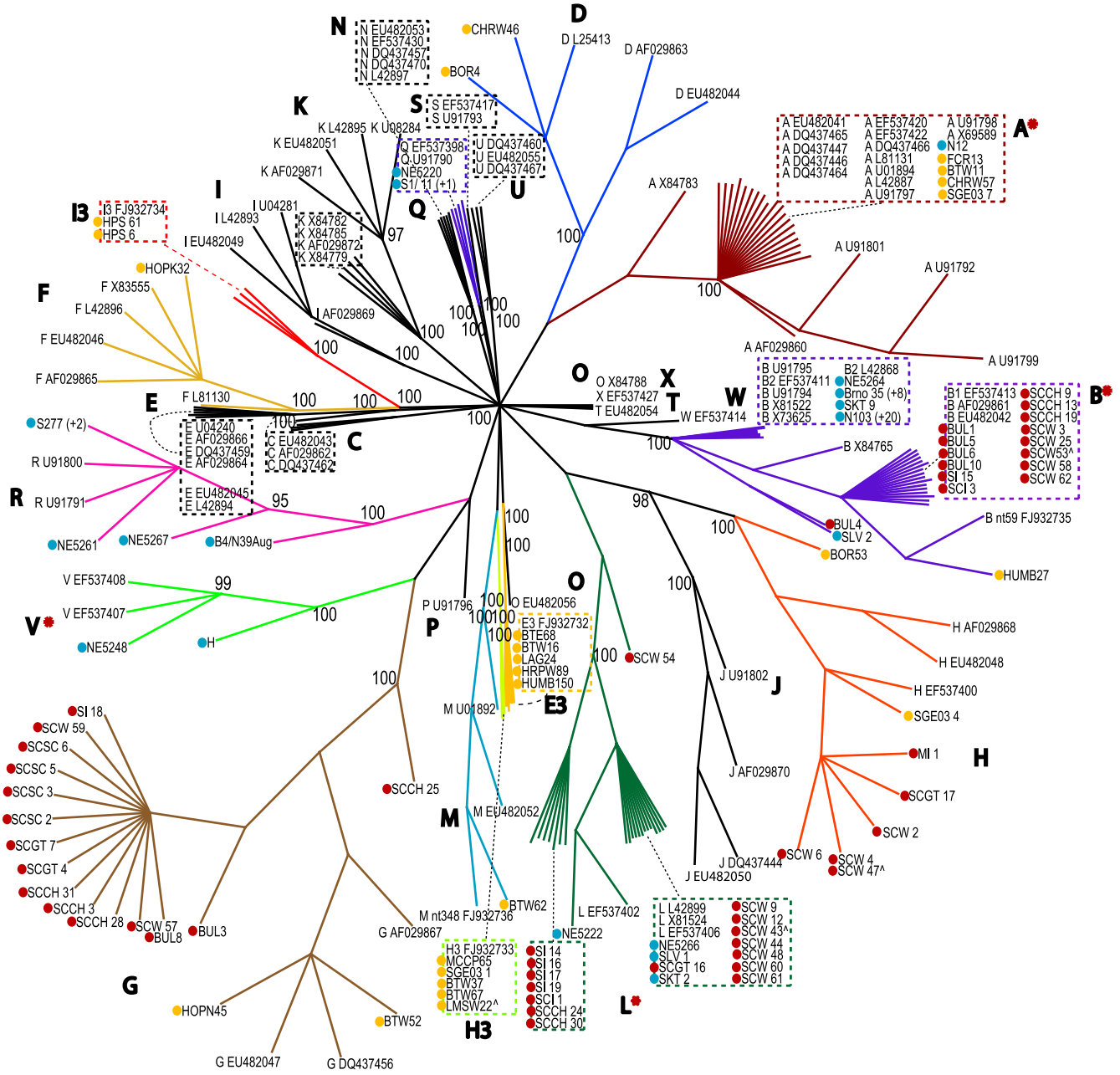

FIG 1 Unrooted neighbor-joining distance tree generated in BioNJ and based on nucleotide sequence alignment of 498-bp fragments of *ospC* genes. Nodes are labeled with the percent bootstrap support when the value is 90% or higher. All clades are marked by capital letters that show the *B. burgdorferi* sensu stricto *ospC* type. All experimental samples in each clade are labeled to indicate the sample origin, as follows: a blue dot in front of a sample name indicates a European origin, a golden dot indicates a Californian origin, and a red dot indicates a southeastern U.S. origin. All unmarked sample names are control samples previously identified as members of an *ospC* type and downloaded from GenBank. Samples with a ^ symbol after their name are those that were not included in the analysis but were placed into clusters with samples whose sequences were identical over the length of the sequence fragment that we were able to obtain for them (see the text). Clades that include experimental *B. burgdorferi* sensu stricto strains isolated from European LD patients are marked with red asterisks.

samples (SCW/SCCH/BUL) clustered with *ospC* allele B strains from New York and Michigan and another consisting of European control and experimental samples.

Eighty percent of *ospC* allele L strains (the second most abundant) were detected among southeastern U.S. *B. burgdorferi* sensu stricto strains cultured from non-human-biting *I. affinis* ticks or from ear clips, bladders, and hearts of local rodent hosts. The remaining 20% of *ospC* allele L strains were isolated from *I. ricinus* nymphs and from the skin of a Slovenian patient (SLV1/JQ236853) diagnosed with acrodermatitis chronica atrophicans (Table 2).

DISCUSSION

Since the recognition of Lyme disease in the 1970s, discussions of it etiology attracted attention of the wide scientific community and the general public. Our analysis of the population structure of *B. burgdorferi* sensu stricto in the southeastern United States rep-

resents the logical extension of similar studies conducted in the northeastern United States, where the disease is highly endemic, and the midwestern United States, where the disease is moderately endemic. The presented results are not meant to be a statistical analysis with an emphasis on the ranking of *Borrelia ospC* alleles but rather an invitation to an open discussion to advance the natural history and understanding of the enzootiology of *B. burgdorferi* sensu stricto in the southeastern United States, previously considered to be a "low-or-no" Lyme disease region.

Even though several different spirochete species cause LD, *B. burgdorferi* sensu stricto is still considered the major species that causes clinical illness in the United States. It also causes LD in Europe although at a lower rate. Molecular analysis revealed an overlap of *B. burgdorferi* sensu stricto genotypes between European and North American spirochete populations (9). While most *B. burgdorferi* sensu lato species or subtypes in Europe are specialized to infect specific taxa of vertebrate hosts ("specialists"), *B. burgdorferi* sensu stricto, as a "generalist," has the ability to infect a wide range of phylogenetically diverse vertebrates. In fact, *B. burgdorferi* sensu lato spirochetes are one of the few groups of zoonotic pathogens for which a molecular mechanism of host "specialism" or "generalism" has been proposed (75).

B. burgdorferi sensu stricto is transmitted from one vertebrate host to another by *Ixodes* sp. ticks belonging to the *Ixodes ricinus* complex. All parasitic stages of these ticks are able to transmit the pathogen, but the nymphal stage appears to be the most important one (47–50). A notable exception is the Asian species *I. persulcatus*, in which the female tick, not the nymph, is a primary vector of *B. burgdorferi* sensu lato. In Europe, *B. burgdorferi* is transmitted by *I. ricinus* ticks (27). In the United States, *I. scapularis* is the primary vector of *B. burgdorferi* sensu stricto in the eastern, northeastern, and north-central regions (2), whereas *I. pacificus* is the primary vector in the far-western part of the United States (51, 52). The majority of LD cases come from the Northeast (>80%) (53), where the population of *I. scapularis* human-biting tick vectors is well established. Lyme disease in the Midwest has received little attention, most probably because the distribution of *I. scapularis* and establishment of a local population in that region were recognized only recently (54), and only around 12% of human LD cases were reported from that region (33). LD in the southeastern United States received even less attention due to the presumably low abundance of *I. scapularis* and the recognition of *Amblyomma americanum*, which does not transmit *B. burgdorferi*, as a major human-biting tick in this region (55). What is needed to be taken into consideration is, as correctly noticed by Stromdahl and Hickling, that "the lack of detection of a tick species is not proof of that species' absence from the survey area" (55). Collection methods are biased for specific tick species, development stages, collection season, and sampling region (J. H. Oliver, Jr., unpublished data). Unfortunately, past tick surveys in the southeastern United States were affected by the amount of efforts in the sampling of different habitats and hosts and the lack of experience with region-specific methodologies. An *I. scapularis* distribution map from 1945 indicated that this tick species was widely distributed in the southeastern United States (56). Even though the southeastern U.S. tick population has undergone dramatic changes due to the increasing wild host population, climate changes, urbanization, or geographical spread, it still does not mean that the southeastern U.S. *I. scapularis* population was decreased so significantly. The rapid expansion of *I. scapularis* ticks in the northeastern United States and the recent invasion of the midwestern United States originated from a very few migrants from the southeastern region after the recession of Pleistocene ice sheets (57). The midwestern tick populations are much younger than the northeastern ones, and both are an order of magnitude younger than the southeastern population of *I. scapularis*. This fact led to the hypothesis that ticks were introduced or reintroduced into new areas by long-distance migration maintained by birds (4, 54, 57–59). As the distribution pattern of *B. burgdorferi* and recolonization of new regions by this pathogen are tightly linked to its tick vectors or vertebrate hosts, we can presume, from one side, that northeastern and midwestern strains of *B. burgdorferi* have the same origin as their main tick vector, *I. scapularis*, which is the southeastern United States. From the other side, the strict connection of the pathogen distribution pattern to the pattern of distribution of its principal vector is naive, as we still do not know how much the pathogen and vector share a common evolutionary or biogeographic history (54, 57).

Several other tick species in the *I. ricinus* complex and those not included in it have been found to maintain *B. burgdorferi* enzootically (2, 4, 51, 60–62). A recent report by Hamer and colleagues showed strong evidences that confirmed the presence of multiple strains of *B. burgdorferi* in areas with an apparent absence of *I. scapularis*, which means an absence of the classical spirochete maintenance cycle of *I. scapularis-P. leucopus* (54). This study supports the previously presented hypotheses of an uncoordinated phylogeography of *B. burgdorferi* and its tick vector *I. scapularis* (57) and the impact of the migratory hosts on pathogen expansion (58, 59). Despite the strong association of LD spirochetes with *I. scapularis*, the population structure, evolutionary history, and biogeography of the pathogen are distinct from those of its arthropod vector (57).

Except for the abundant tick vector, appropriate vertebrate hosts are required for enzootic maintenance of *B. burgdorferi*. There is a variety of vertebrate hosts, including small mammals and birds, that might serve as reservoir hosts for *B. burgdorferi* in the United States. However, in general, rodents appear to be the most common reservoir hosts in the North American regions where LD is endemic (2, 28, 34, 52, 63, 64). Recent studies suggested that migration of infected vertebrate hosts may have a larger impact on the contemporary expansion of the pathogen population than the movement of tick vectors (54, 57). The low prevalence of *B. burgdorferi* in *I. scapularis* does not necessarily mean that there is a low prevalence or an absence of the pathogen in the region, taking into consideration the existence of "cryptic" maintenance cycles or the impact of migrating infected reservoir hosts (54). A convincing scenario showing how migrating hosts may accelerate the increasing risk of LD to humans through the maintenance of *B. burgdorferi* in the absence of classic *I. scapularis-P. leucopus* transmission was recently presented by Hamer and colleagues (54).

An association between LD severity and *ospC* alleles was reported previously (9, 50, 65, 66). Twenty-eight *ospC* alleles have been identified in *B. burgdorferi* sensu stricto (67). While some spirochete complexes were believed to be restricted exclusively to North America (genotypes B1, C, D, F, G, H, I, J, N, and U) or exclusively to Europe (genotypes B2, S, L, Q, and V), three *ospC* types (A, E, and K) were previously detected on both continents. Furthermore, the sequences of the isolates were identical, suggesting that each group was able to thrive in a new niche consisting of novel vector and host species with little or no genetic change (9). To date, four *ospC* alleles, A, B, I, and K, are responsible for sys-

temic LD in humans around the world (9, 22). Additional genotypes, C, D, N, F, H, E, G, and M, have been found in disseminated sites (18–21). Some of the *ospC* alleles that correlate with human invasiveness were recently detected in the southeastern United States, where the disease is not endemic.

LD is increasing in incidence and is spreading geospatially. Approximately 85,000 Lyme disease cases are estimated in Europe every year (68). Nearly 30,000 confirmed cases of LD were reported in 2009 in the United States, in addition to another 8,500 probable cases (http://www.cdc.gov/lyme/stats/chartstables/reportedcases_statelocality.html). Taking into consideration the significant number of underreported cases, the total annual number of LD cases in the world might be as high as 255,000 (69). The spreading or exchange of highly pathogenic spirochete clones between continents might be supported by the transoceanic migration of host species, especially birds.

Analysis of 53 *B. burgdorferi* sensu stricto strains cultivated from tick vectors and rodent hosts from the southeastern United States revealed that 30% of isolates were *ospC* allele L strains, a type previously considered to be exclusively European (9). Most of the samples were cultured from *I. affinis*, ticks that usually do not bite humans, thus playing little if any role in the direct transmission of *B. burgdorferi* to humans, as well as from ear clips or bladders of two major reservoir hosts of *B. burgdorferi* in the southeastern United States, *Peromyscus gossypinus* and *Sigmodon hispidus*. *ospC* allele L shared a frequency of distribution with *ospC* allele B strains (30.2% each) in the southeastern United States. Two other *ospC* alleles detected among the 53 *B. burgdorferi* strains were alleles G and H (28.3% and 11.3%, respectively) (70). It was believed that *ospC* allele L very rarely, if ever, causes human disease (9, 18, 22). Concerning the infectivity to nonhuman species, it was previously found that *B. burgdorferi ospC* allele L strains are not infectious to four principal reservoir host species in the northeastern region of hyperendemicity, *Peromyscus leucopus* (white-footed mouse), *Tamias striatus* (eastern chipmunk), *Blarina brevicauda* (short-tailed shrew), and *Sciurus carolinensis* (gray squirrel) (17). However, as analyzed in this study, *B. burgdorferi ospC* allele L strains showed the ability to disseminate in two of the most common natural reservoir hosts in the South, the cotton mouse (*P. gossypinus*) and the cotton rat (*Sigmodon hispidus*). It is possible that the interspecific variation in the vertebrate immune system may provide resistance to infection by certain *ospC* alleles (70). The limited distribution of both primary reservoir hosts of *B. burgdorferi* sensu stricto *ospC* type L strains, the cotton mouse *P. gossypinus* and the cotton rat *S. hispidus*, and the knowledge that they are parasitized by *I. scapularis*, *I. affinis*, *I. minor*, *Dermacentor variabilis*, and *Amblyomma maculatum* (2) suggest that globally rare *ospC* allele L might be limited largely to the southeastern United States. This conclusion is indirectly supported by previous work by Anderson and Norris (71). Studying the genetic diversity of *B. burgdorferi* in *P. leucopus* in southern Maryland, those authors found 5 different *ospC* types among Maryland samples: alleles A, B, G, H, and K. Southern Maryland represents the border between the regions of distribution of *P. leucopus* and *P. gossypinus*. While *ospC* alleles B, G, and H are present in the southeastern states of the United States and in southern Maryland, *ospC* allele L is restricted to the southeastern part only, most probably confirming the strong host specificity of this *ospC* allele for local rodent hosts. At the same time, *ospC* alleles A and K are present only in regions of distribution of *P. leucopus* and are absent in the Southeast, where *P. gossypinus* replaces *P. leucopus*.

Detection of the invasive *ospC* type B in 30% of samples was unexpected for strains from the southeastern United States. This raises the question of whether the risk of LD to humans in this region has been overlooked or if the geographic distribution of the LD spirochete has evolved over time. In order for LD to occur, humans must be exposed to invasive strains via a tick bite. However, the above-mentioned *ospC* allele B was detected in spirochete strains isolated from either rodent hosts or tick vectors that rarely bite humans. Previous analyses of non-human-biting *I. affinis* ticks from the southeastern United States revealed that they are heavily infected with *B. burgdorferi* (33 to 35%) (72, 73). Most probably, maintenance vectors such as *I. affinis* could have a significant impact on Lyme disease dynamics, helping to maintain high levels of *B. burgdorferi* in reservoir hosts that are later fed upon by bridge vectors that often bite humans (2, 57).

It will also be interesting to determine how much the structure of the *B. burgdorferi* sensu stricto population has changed in Europe. Is it still correct that *B. burgdorferi* sensu stricto is a strain of minor importance in this part of the world? How much has the pattern of distribution of this species in Europe changed over time, and if it has changed, what are the factors contributing to such changes?

Of 4 *ospC* alleles, B, G, H, and N, that have been detected in LD patients in the northeastern and midwestern United States, 3 alleles, B, G, and H, at a lower rate, are widely distributed in the southeastern part of the country and are associated with rodent hosts or non-human-biting ticks. *ospC* allele B, widely distributed in the Northeast and Midwest, is commonly associated with disseminated LD around the world. Together with *ospC* alleles L and G, it became the most frequent *ospC* allele among *B. burgdorferi* sensu stricto strains from the southeastern United States. *ospC* allele H, commonly detected in LD patients from the northeastern and midwestern United States, seems to be the fourth most frequently detected *ospC* allele in the Southeast. Isolation of *ospC* allele H strains from secondary sites of host infection may suggest its potential to develop invasive disease. This is in agreement with previous results obtained with human and murine isolates (18).

This is the first expanded *ospC* genotyping survey of *B. burgdorferi* sensu stricto strains from the southeastern United States. Although *B. burgdorferi* sensu stricto is endemic in many foci over large areas of the southeastern United States, relatively few human cases have been reported from this region. The lower prevalence of LD in the southeastern United States was previously attributed to (i) a parallel cycle involving non-human-biting maintenance vectors, a "cryptic cycle"; (ii) variations in the vertebrate immune system that provide resistance to infection by certain strains; or (iii) different subsets of *B. burgdorferi* sensu stricto lineages that are present in different regions of the United States (2, 74). This could be determined by differences in the enzootiology of *B. burgdorferi* in the southeastern United States, which differs fundamentally from that reported for the northeastern United States and Europe. Lyme disease in the southeastern United States might not be a public health problem, but it deserves closer attention as a curious natural event that has all prerequisites, pathogen, competent tick vectors, and an array of reservoir hosts, to develop into a medical problem, turning from an enzootic to a zoonotic system. A detailed preface to an offered discussion about Lyme disease in the southeastern United States (2) is now supported by additional

laboratory results. Zoonotic diseases such as LD become a concern when they spill over into the human population. It might be endemic but not yet recognized unless humans become ill and are accurately diagnosed (2). Taking into consideration the changes that have occurred in nature and in human society and the substantial amount of new information concerning the global distribution of LD and its growing list of causative agents, it is time to take a fresh look at LD in the southeastern United States.

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REFERENCES

1. **Kurtenbach K, Hanincová K, Tsao JI, Margos G, Fish D, Ogden NH.** 2006. Fundamental processes in the evolutionary ecology of Lyme borreliosis. Nat. Rev. Microbiol. **4**:660–669.
2. **Oliver JH, Jr.** 1996. Lyme borreliosis in the southern United States: a review. J. Parasitol. **82**:926–935.
3. **Comstedt P, Bergström S, Olsen B, Garpmo U, Marjavaara L, Mejlon H, Barbour AG, Bunikis J.** 2006. Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. Emerg. Infect. Dis. **12**:1087–1095.
4. **Olsen B, Duffy DC, Jaenson TG, Gylfe A, Bonnedahl J, Bergström S.** 1995. Transhemispheric exchange of Lyme disease spirochetes by seabirds. J. Clin. Microbiol. **33**:3270–3274.
5. **Poupon M-A, Lommano E, Humair PF, Douet V, Rais O, Schaad M, Jenni L, Gern L.** 2006. Prevalence of *Borrelia burgdorferi* sensu lato in ticks collected from migratory birds in Switzerland. Appl. Environ. Microbiol. **72**:976–979.
6. **Foretz M, Postic D, Baranton G.** 1997. Phylogenetic analysis of *Borrelia burgdorferi* sensu stricto by arbitrarily primed PCR and pulsed-field gel electrophoresis. Int. J. Syst. Bacteriol. **47**:11–18.
7. **Marti Ras N, Postic D, Foretz M, Baranton G.** 1997. *Borrelia burgdorferi* sensu stricto, a bacterial species "made in the USA"? Int. J. Syst. Bacteriol. **47**:1112–1117.
8. **Postic D, Ras NM, Lane RS, Humair P, Wittenbrink MM, Baranton G.** 1999. Common ancestry of *Borrelia burgdorferi* sensu lato strains from North America and Europe. J. Clin. Microbiol. **37**:3010–3012.
9. **Qiu WG, Bruno JF, McCaig WD, Xu Y, Livey I, Schriefer ME, Luft BJ.** 2008. Wide distribution of a high-virulence *Borrelia burgdorferi* clone in Europe and North America. Emerg. Infect. Dis. **14**:1097–1104.
10. **Girard YA, Fedorova N, Lane RS.** 2011. Genetic diversity of *Borrelia burgdorferi* and detection of *B. bissettii*-like DNA in serum of north-coastal California residents. J. Clin. Microbiol. **49**:945–954.
11. **Rudenko N, Golovchenko M, Grubhoffer L, Oliver JH, Jr.** 2011. Updates on *Borrelia burgdorferi* sensu lato complex with respect to public health. Ticks Tick Borne Dis. **2**:123–128.
12. **Rudenko N, Golovchenko M, Mokrácek A, Piskunová N, Ruzek D, Mallatová N, Grubhoffer L.** 2008. Detection of *Borrelia bissettii* in cardiac valve tissue of a patient with endocarditis and aortic valve stenosis in the Czech Republic. J. Clin. Microbiol. **46**:3540–3543.
13. **Rudenko N, Golovchenko M, Rùzek D, Piskunova N, Mallátová N, Grubhoffer L.** 2009. Molecular detection of *Borrelia bissettii* DNA in serum samples from patients in the Czech Republic with suspected borreliosis. FEMS Microbiol. Lett. **292**:274–281.
14. **Liveris D, Gazumyan A, Schwartz I.** 1995. Molecular typing of *Borrelia burgdorferi* sensu lato by PCR-restriction fragment length polymorphism analysis. J. Clin. Microbiol. **33**:589–595.
15. **Liveris D, Varde S, Iyer R, Koenig S, Bittker S, Cooper D, McKenna D, Nowakowski J, Nadelman RB, Wormser GP, Schwartz I.** 1999. Genetic diversity of *Borrelia burgdorferi* in Lyme disease patients as determined by culture versus direct PCR with clinical specimens. J. Clin. Microbiol. **37**:565–569.
16. **Wang IN, Dykhuizen DE, Qiu W, Dunn JJ, Bosler EM, Luft BJ.** 1999. Genetic diversity of *ospC* in a local population of *Borrelia burgdorferi* sensu stricto. Genetics **151**:15–30.
17. **Brisson D, Dykhuizen DE.** 2004. OspC diversity in *Borrelia burgdorferi*: different hosts are different niches. Genetics **168**:713–722.
18. **Alghaferi MY, Anderson JM, Park J, Auwaerter PG, Aucott JN, Norris DE, Dumler JS.** 2005. *Borrelia burgdorferi ospC* heterogeneity among human and murine isolates from a defined region of northern Maryland and southern Pennsylvania: lack of correlation with invasive and noninvasive genotypes. J. Clin. Microbiol. **43**:1879–1884.
19. **Brisson D, Baxamusa N, Schwartz I, Wormser GP.** 2011. Biodiversity of *Borrelia burgdorferi* strains in tissues of Lyme disease patients. PLoS One **6**:e22926. doi:10.1371/journal.pone.0022926.
20. **Dykhuizen DE, Brisson D, Sandigursky S, Wormser GP, Nowakowski J, Nadelman RB, Schwartz I.** 2008. The propensity of different *Borrelia burgdorferi* sensu stricto genotypes to cause disseminated infections in humans. Am. J. Trop. Med. Hyg. **78**:806–810.
21. **Ivanova L, Christova I, Neves V, Aroso M, Meirelles L, Brisson D, Gomes-Solecki M.** 2009. Comprehensive seroprofiling of sixteen *B. burgdorferi* OspC: implications for Lyme disease diagnostics design. Clin. Immunol. **132**:393–400.
22. **Seinost G, Dykhuizen DE, Dattwyler RJ, Golde WT, Dunn JJ, Wang IN, Wormser GP, Schriefer ME, Luft BJ.** 1999. Four clones of *Borrelia burgdorferi* sensu stricto cause invasive infection in humans. Infect. Immun. **67**:3518–3524.
23. **Wang G, Ojaimi C, Iyer R, Saksenberg V, McClain SA, Wormser GP, Schwartz I.** 2001. Impact of genotypic variation of *Borrelia burgdorferi* sensu stricto on kinetics of dissemination and severity of disease in C3H/HeJ mice. Infect. Immun. **69**:4303–4312.
24. **Wang G, Ojaimi C, Wu H, Saksenberg V, Iyer R, Liveris D, McClain SA, Wormser GP, Schwartz I.** 2002. Disease severity in a murine model of Lyme borreliosis is associated with the genotype of the infecting *Borrelia burgdorferi* sensu stricto strain. J. Infect. Dis. **186**:782–791.
25. **Anderson JF.** 1988. Mammalian and avian reservoirs for *Borrelia burgdorferi*. Ann. N. Y. Acad. Sci. **539**:180–191.
26. **Anderson JF.** 1989. Ecology of Lyme disease. Conn. Med. **53**:343–346.
27. **Gern L.** 2008. *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis: life in the wilds. Parasite **15**:244–247.
28. **Lane RS, Piesman J, Burgdorfer W.** 1991. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annu. Rev. Entomol. **36**:587–609.
29. **Qiu WG, Bosler EM, Campbell JR, Ugine GD, Wang IN, Luft BJ, Dykhuizen DE.** 1997. A population genetic study of *Borrelia burgdorferi* sensu stricto from eastern Long Island, New York, suggested frequency-dependent selection, gene flow and host adaptation. Hereditas **127**:203–216.
30. **Theisen M, Borre M, Mathiesen MJ, Mikkelsen B, Lebech AM, Hansen K.** 1995. Evolution of the *Borrelia burgdorferi* outer surface protein OspC. J. Bactriol. **177**:3036–3044.
31. **Wang G, van Dam AP, Dankert J.** 1999. Evidence for frequent *ospC* gene transfer between *Borrelia valaisiana* sp. nov. and other Lyme disease spirochetes. FEMS Microbiol. Lett. **177**:289–296.
32. **Bunikis J, Garpmo U, Tsao J, Berglund J, Fish D, Barbour AG.** 2004. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. Microbiology **150**:1741–1755.
33. **Qiu WG, Dykhuizen DE, Acosta MS, Luft BJ.** 2002. Geographic uniformity of the Lyme disease spirochete (*Borrelia burgdorferi*) and its shared history with tick vector (*Ixodes scapularis*) in the Northeastern United States. Genetics **160**:833–849.
34. **Barbour AG, Fish D.** 1993. The biological and social phenomenon of Lyme disease. Science **260**:1610–1616.
35. **O'Connell S.** 2011. Lyme borreliosis and other ixodid tick-borne diseases—a European perspective, p. 405–435. *In* Critical needs and gaps in under-

standing prevention, amelioration, and resolution of Lyme and other tick-borne diseases: the short-term and long-term outcomes. National Academies Press, Washington, DC.
36. **Piesman J, Gern L.** 2004. Lyme borreliosis in Europe and North America. Parasitology **129**(Suppl):S191–S220. doi:10.1017/S0031182003004694.
37. **Rudenko N, Golovchenko M, Grubhoffer L, Oliver JH, Jr.** 2011. *Borrelia carolinensis* sp. nov., a new species of *Borrelia burgdorferi* sensu lato complex isolated from rodents and a tick from the south-eastern USA. Int. J. Syst. Evol. Microbiol. **61**:381–383.
38. **Rudenko N, Golovchenko M, Lin T, Gao L, Grubhoffer L, Oliver JH, Jr.** 2009. Delineation of a new species of the *Borrelia burgdorferi* sensu lato complex, *Borrelia americana* sp. nov. J. Clin. Microbiol. **47**:3875–3880.
39. **Stanek G, Fingerle V, Hunfeld KP, Jaulhac B, Kaiser R, Krause A, Kristoferitsch W, O'Connell S, Ornstein K, Strle F, Gray J.** 2011. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin. Microbiol. Infect. **17**:69–79.
40. **Hubalek Z.** 2009. Epidemiology of Lyme borreliosis. Curr. Probl. Dermatol. **37**:31–50.
41. **Steere AC, Coburn J, Glickstein L.** 2004. The emergence of Lyme disease. J. Clin. Invest. **113**:1093–1101.
42. **Girard YA, Travinsky B, Schotthoefer A, Fedorova N, Eisen RJ, Eisen L, Barbour AG, Lane RS.** 2009. Population structure of the Lyme borreliosis spirochete *Borrelia burgdorferi* in the western black-legged tick (*Ixodes pacificus*) in Northern California. Appl. Environ. Microbiol. **75**:7243–7252.
43. **Rudenko N, Golovchenko M, Grubhoffer L, Oliver JH, Jr.** 2009. *Borrelia carolinensis* sp. nov., a new (14th) member of the *Borrelia burgdorferi* sensu lato complex from the southeastern region of the United States. J. Clin. Microbiol. **47**:134–141.
44. **Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG.** 2007. Clustal W and Clustal X version 2.0. Bioinformatics **23**:2947–2948.
45. **Brisson D, Vandermause MF, Meece JK, Reed KD, Dykhuizen DE.** 2010. Evolution of northeastern and midwestern *Borrelia burgdorferi*, United States. Emerg. Infect. Dis. **16**:911–917.
46. **Gascuel O.** 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Mol. Biol. Evol. **14**:685–695.
47. **Anderson JF, Magnarelli LA, Stafford KC, III.** 1990. Bird-feeding ticks transstadially transmit *Borrelia burgdorferi* that infect Syrian hamsters. J. Wildl. Dis. **26**:1–10.
48. **Danielová V, Daniel M, Schwarzová L, Materna J, Rudenko N, Golovchenko M, Holubová J, Grubhoffer L, Kilián P.** 2010. Integration of a tick-borne encephalitis virus and *Borrelia burgdorferi* sensu lato into mountain ecosystems, following a shift in the altitudinal limit of distribution of their vector, *Ixodes ricinus* (Krkonose mountains, Czech Republic). Vector Borne Zoonotic Dis. **10**:223–230.
49. **Kurtenbach K, Peacey M, Rijpkema SG, Hoodless AN, Nuttall PA, Randolph SE.** 1998. Differential transmission of the genospecies of *Borrelia burgdorferi* sensu lato by game birds and small rodents in England. Appl. Environ. Microbiol. **64**:1169–1174.
50. **Scott JD, Lee MK, Fernando K, Durden LA, Jorgensen DR, Mak S, Morshed MG.** 2010. Detection of Lyme disease spirochete, Borrelia burgdorferi sensu lato, including three novel genotypes in ticks (Acari: Ixodidae) collected from songbirds (Passeriformes) across Canada. J. Vector Ecol. **35**:124–139.
51. **Brown RN, Lane RS.** 1992. Lyme disease in California: a novel enzootic transmission cycle of *Borrelia burgdorferi*. Science **256**:1439–1442.
52. **Lane RS, Loye JE.** 1991. Lyme disease in California: interrelationship of ixodid ticks (Acari), rodents, and *Borrelia burgdorferi*. J. Med. Entomol. **28**:719–725.
53. **Orloski KA, Hayes EB, Campbell GL, Dennis DT.** 2000. Surveillance for Lyme disease—United States, 1992-1998. MMWR CDC Surveill. Summ. **49**:1–11.
54. **Hamer SA, Hickling GJ, Sidge JL, Rosen ME, Walker ED, Tsao JI.** 2011. Diverse *Borrelia burgdorferi* strains in a bird-tick cryptic cycle. Appl. Environ. Microbiol. **77**:1999–2007.
55. **Stromdahl E, Hickling G.** 2012. Beyond Lyme: aetiology of tick-borne human diseases with emphasis on the South-Eastern United States. Zoonoses Public Health **59**:48–64.
56. **Bishopp FC, Trembley HL.** 1945. Distribution and hosts of certain North American ticks. J. Parasitol. **31**:1–54.
57. **Humphrey PT, Caporale DA, Brisson D.** 2010. Uncoordinated phylogeography of *Borrelia burgdorferi* and its tick vector, *Ixodes scapularis*. Evolution **64**:2653–2663.
58. **Ogden NH, Lindsay LR, Hanincová K, Barker IK, Bigras-Poulin M, Charron DF, Heagy A, Francis CM, O'Callaghan CJ, Schwartz I, Thompson RA.** 2008. Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. Appl. Environ. Microbiol. **74**:1780–1790.
59. **Ogden NH, Margos G, Aanensen DM, Drebot MA, Feil EJ, Hanincová K, Schwartz I, Tyler S, Lindsay LR.** 2011. Investigation of genotypes of *Borrelia burgdorferi* in *Ixodes scapularis* ticks collected during surveillance in Canada. Appl. Environ. Microbiol. **77**:3244–3254.
60. **Anderson JF, Magnarelli LA, LeFebvre RB, Andreadis TG, McAninch JB, Perng GC, Johnson RC.** 1989. Antigenically variable *Borrelia burgdorferi* isolated from cottontail rabbits and *Ixodes dentatus* in rural and urban areas. J. Clin. Microbiol. **27**:13–20.
61. **Gern L, Rouvinez E, Toutoungi LN, Godfroid E.** 1997. Transmission cycles of *Borrelia burgdorferi* sensu lato involving *Ixodes ricinus* and/or *I. hexagonus* ticks and the European hedgehog, *Erinaceus europaeus*, in suburban and urban areas in Switzerland. Folia Parasitol. (Praha) **44**:309–314.
62. **Oliver JH, Jr, Chandler FW, Jr, James AM, Huey LO, Vogel GN, Sanders FH, Jr.** 1996. Unusual strain of *Borrelia burgdorferi* isolated from *Ixodes dentatus* in central Georgia. J. Parasitol. **82**:936–940.
63. **Oliver JH, Jr, Chandler FW, Jr, James AM, Sanders FH, Jr, Hutcheson HJ, Huey LO, McGuire BS, Lane RS.** 1995. Natural occurrence and characterization of the Lyme disease spirochete, *Borrelia burgdorferi*, in cotton rats (*Sigmodon hispidus*) from Georgia and Florida. J. Parasitol. **81**:30–36.
64. **Oliver JH, Jr, Chandler FW, Jr, Luttrell MP, James AM, Stallknecht DE, McGuire BS, Hutcheson HJ, Cummins GA, Lane RS.** 1993. Isolation and transmission of the Lyme disease spirochete from the southeastern United States. Proc. Natl. Acad. Sci. U. S. A. **90**:7371–7375.
65. **Hanincová K, Liveris D, Sandigursky S, Wormser GP, Schwartz I.** 2008. *Borrelia burgdorferi* sensu stricto is clonal in patients with early Lyme borreliosis. Appl. Environ. Microbiol. **74**:5008–5014.
66. **Strle K, Jones KL, Drouin EE, Li X, Steere AC.** 2011. *Borrelia burgdorferi* RST1 (OspC type A) genotype is associated with greater inflammation and more severe Lyme disease. Am. J. Pathol. **178**:2726–2739.
67. **Barbour AG, Travinsky B.** 2010. Evolution and distribution of the *ospC* gene, a transferable serotype determinant of *Borrelia burgdorferi*. mBio **1**(4):e00153–10. doi:10.1128/mBio.00153-10.
68. **Lindgren E, Jaenson TGT.** 2006. Lyme borreliosis in Europe: influences of climate and climate change, epidemiology and adaptation measures, p 157–188. *In* Menne B, Ebi KL (ed), Climate change and adaptation strategies for human health. Steinkopff, Darmstadt, Germany.
69. **Campbell GL, Fritz CL, Fish D, Nowakowski J, Nadelman RB, Wormser GP.** 1998. Estimation of the incidence of Lyme disease. Am. J. Epidemiol. **148**:1018–1026.
70. **Rudenko N, Golovchenko M, Grubhoffer L, Oliver JH, Jr.** 2013. The rare *ospC* allele L of *Borrelia burgdorferi* sensu stricto, commonly found among samples collected in a coastal plain area of the southeastern United States, is associated with *Ixodes affinis* ticks and local rodent hosts *Peromyscus gossypinus* and *Sigmodon hispidus*. Appl. Environ. Microbiol. **79**:1403–1406.
71. **Anderson JM, Norris DE.** 2006. Genetic diversity of *Borrelia burgdorferi* sensu stricto in *Peromyscus leucopus*, the primary reservoir of Lyme disease in a region of endemicity in southern Maryland. Appl. Environ. Microbiol. **72**:5331–5341.
72. **Harrison BA, Rayburn WH, Jr, Toliver M, Powell EE, Engber BR, Durden LA, Robbins RG, Prendergast BF, Whitt PB.** 2010. Recent discovery of widespread *Ixodes affinis* (Acari: Ixodidae) distribution in North Carolina with implications for Lyme disease studies. J. Vector Ecol. **35**:174–179.
73. **Maggi RG, Reichelt S, Toliver M, Engber B.** 2010. Borrelia species in *Ixodes affinis* and *Ixodes scapularis* ticks collected from the coastal plain of North Carolina. Ticks Tick Borne Dis. **1**:168–171.
74. **Lane RS, Quistad GB.** 1998. Borreliacidal factor in the blood of the western fence lizard (*Sceloporus occidentalis*). J. Parasitol. **84**:29–34.
75. **Woolhouse ME, Taylor LH, Haydon DT.** 2001. Population biology of multihost pathogens. Science **292**:1109–1112.