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Genetic Diversity of the Outer Surface Protein C Gene of Southern *Borrelia* Isolates and Its Possible Epidemiological, Clinical, and Pathogenetic Implications

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The *ospC* genes of 20 southern *Borrelia* strains were sequenced. The strains consisted of *B. burgdorferi* sensu stricto, *B. andersonii*, *B. bissettii*, one undescribed genospecies, MI-8, and one probably new *Borrelia* species, TXW-1. A high degree of similarity exists between *B. burgdorferi* sensu stricto and *B. bissettii* and between *B. bissettii* and *B. andersonii*. Lateral transfers of the *ospC* gene probably occurred between *B. burgdorferi* sensu stricto and *B. bissettii* and between *B. bissettii* and *B. andersonii*. Internal gene recombination appears to occur among them. The highest degree of genetic diversity among them was observed in the two variable domains (V1 and V2), semivariable domain (SV), and the species-specific epitopes (between amino acids 28 and 31). Differences in *ospC* sequences among southern strains reflect diversity at the strain and genospecies levels. MI-8, which was recognized as an undescribed genospecies in our previous reports, remains distinguishable in our current analysis of *ospC* genes and is distinct from *B. burgdorferi* sensu stricto. Interestingly, another undescribed southern isolate, TXW-1, was not amplified under various PCR conditions. Compared to European *B. burgdorferi* sensu stricto strains, American *B. burgdorferi* sensu stricto strains show greater genetic heterogeneity. Southern *B. burgdorferi* sensu stricto, *B. andersonii*, and *B. bissettii* isolates were intermixed with each other in the phylogenetic trees. In the derived trees in our work, at least one southeastern strain of *B. burgdorferi*, MI-2, most closely aligns with a so-called invasive cluster that possesses many proven human-invasive strains. Transmission experiments show that MI-2 and the strains in this group of southern spirochetes are able to infect mice and hamsters and that the typical vector of Lyme disease, *Ixodes scapularis*, can acquire the spirochetes from infected mammals. Currently, strain MI-2 appears to be the only southern isolate among the 20 we analyzed that clusters with an OspC invasive group and thus might be invasive for humans.

Lyme disease is the most common vector-borne disease in humans in North America and Eurasia. There were 122,651 Lyme disease cases reported from 50 states and the District of Columbia from 1990 through 1999 in the United States (17), and about 50,000 cases are estimated to occur in Europe annually (40). Human Lyme disease may involve multiple organs or tissues, resulting in skin, cardiac, neurological, and musculoskeletal disorders. Multiple erythema migrans and Lyme arthritis are more common in the United States than in Europe, whereas neuroborreliosis occurs frequently in European patients. Borrelial lymphocytoma and acrodermatitis chronica atrophicans are well documented in European Lyme disease patients but rarely recognized among Lyme disease patients in the United States.

The causative agent of Lyme disease, *Borrelia burgdorferi* sensu lato, is genetically highly divergent (8, 30, 35, 36, 40, 49; T. Lin, J. H. Oliver, Jr., T. M. Kollars, Jr., and K. L. Clark, Proc. VIII Int. Conf. Lyme Borreliosis Other Emerging Tick-Borne Dis., p. 8, 1999). Different *Borrelia* genospecies sometimes have been associated with distinct clinical manifestations of Lyme disease, although there is often an overlap of symptoms. Lyme arthritis is often related to *B. burgdorferi* sensu stricto, neuroborreliosis is often associated with *B. garinii*, and acrodermatitis chronica atrophicans may occur in Lyme disease patients infected with *B. afzelii* (5, 8, 13, 39, 47, 66).

Eleven genospecies or genomic groups within the *B. burgdorferi* sensu lato complex have been described based on their phenotypic and genetic characteristics since the discovery of *B. burgdorferi* in 1982 (11) and its subsequent description (32). Three of them, *B. burgdorferi* sensu stricto (8), *B. andersonii* (6, 37, 47), and *B. bissettii* (6, 47, 48), have been identified in the United States. *B. burgdorferi* sensu stricto is widely distributed in the northeastern, southeastern, mid-Atlantic, and north central states and northern California (17, 35; Lin et al., Proc. VIII Int. Conf. Lyme Borreliosis Other Emerg. Tick-Borne Dis.). It is the only genospecies of *B. burgdorferi* sensu lato that has been cultured from humans with Lyme borreliosis in North America. *B. andersonii* and *B. bissettii* were isolated mainly from ticks and small mammals from New York (2, 4), California (48), Colorado (53), and the southeastern United States (35, 39; Lin et al., Proc. VIII Int. Conf. Lyme Borreliosis Other Emerg. Tick-Borne Dis.). *B. andersonii* occurs in the eastern half of the United States and appears to exist primarily in an enzootic cycle involving cottontail rabbits and *Ixodes dentatus.* *B. bissettii* occurs in the western and southern United States but infrequently in the northern region. It appears to be maintained in several enzootic transmission cycles in California and the southeastern United States and involves several tick species, including *Ixodes spinipalpis* and the human-biting *I. pacificus* in the west, and *Ixodes minor* and the human-biting *I. scapularis* in the eastern half of the United States (10, 42; J. H.

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TABLE 1. Origins of *B. burgdorferi* sensu lato strains in this study

Isolate	Host	Source	Location	Passage no.	Genospecies
MI-2	*Peromyscus gossypinus*	Bladder, ear clip	Merritt Island, Brevard County, Fla.	3	*Borrelia burgdorferi* sensu stricto
MI-5	*Sigmodon hispidus*	Bladder, ear clip	Merritt Island, Brevard County, Fla.	3	*Borrelia burgdorferi* sensu stricto
SI-1	*Peromyscus gossypinus*	Bladder	Sapelo Island, McIntosh County, Ga.	3	*Borrelia burgdorferi* sensu stricto
SM-1	*Peromyscus gossypinus*	Ear clip	St. Marys, Camden County, Ga.	3	*Borrelia burgdorferi* sensu stricto
SCI-2	*Peromyscus gossypinus*	Ear clip	St. Catherines Island, Liberty County, Ga.	3	*Borrelia burgdorferi* sensu stricto
MI-6	*Sigmodon hispidus*	Ear clip	Merritt Island, Brevard County, Fla.	3	*Borrelia bissettii*
MI-9	*Peromyscus gossypinus*	Ear clip	Merritt Island, Brevard County, Fla.	3	*Borrelia bissettii*
SCW-30h	*Ixodes minor* nymph	Carolina wren	Wedge Plantation, Charleston County, S.C.	3	*Borrelia bissettii*
SCGT-8a	*Ixodes minor* male	Woodrat	Georgetown County, S.C.	3	*Borrelia bissettii*
SCGT-10	*Neotoma floridana*	Ear clip	Georgetown County, S.C.	3	*Borrelia bissettii*
AI-1	*Sigmodon hispidus*	Bladder, ear clip	Amelia Island, Nassau County, Fla.	3	*Borrelia bissettii*
FD-1	*Sigmodon hispidus*	Bladder, ear clip	Favor Dykes, Flagler County, Fla.	3	*Borrelia bissettii*
MOK-3a	*Ixodes dentatus* nymph	Rabbit	Koch Farm, Bollinger County, Mo.	3	*Borrelia andersonii*
MOD-1	2 *Ixodes dentatus* nymphs	Rabbit	Dowd Farm, Bollinger County, Mo.	3	*Borrelia andersonii*
MOD-5	*Ixodes dentatus* nymph	Rabbit	Dowd Farm, Bollinger County, Mo.	3	*Borrelia andersonii*
MOS-1b	*Ixodes dentatus* larva	Rabbit	Swinton, Stoddard County, Mo.	3	*Borrelia andersonii*
SI-10	*Ixodes scapularis* female	Drag	Sapelo Island, McIntosh County, Ga.	3	*Borrelia andersonii*
BC-1	*Ixodes dentatus* nymph	Drag	Macon, Bibb County, Ga.	3	*Borrelia andersonii*
MI-8	*Sigmodon hispidus*	Ear clip	Merritt Island, Brevard County, Fla.	3	*Borrelia* sp.
TXW-1	*Dermacentor variabilis* male	Coyote	Webb County, Tex.	3	*Borrelia* sp.

Oliver, Jr., Proc. II Int. Symp. Lyme Dis. Emerg. Re-emerg. Dis., p. 83–94, 1997).

Various phenotypic and genotypic techniques have been used to study variability and to identify genospecies in the *B. burgdorferi* sensu lato complex. Analysis of the *ospC* gene has yielded valuable information. The *ospC* gene, located in the 26-kb circular plasmid, has a high degree of heterogeneity (30, 51, 68). Its diversity has been described among and within genospecies (29, 30, 68, 70), and lateral transfer of the *ospC* gene and internal gene recombination are reported to increase the variability among *ospC* sequences (20, 30, 38). Interestingly, the divergent sequences of *ospC* and the deduced amino acid sequences reported from *B. burgdorferi* sensu lato have been correlated with the distribution of the genospecies (29, 68). A species-specific motif was found in the conserved amino terminus of the OspC protein (30, 70), and intergenic transfer and internal recombination have not been reported in the motif region except for a few *B. valaisiana* isolates, which might have exchanged a complete *ospC* gene from *B. garinii* or *B. afzelii* (63).

Sequence analysis of *ospC* and its deduced amino acids appears to be a useful tool for identification of *Borrelia* species and for evaluating the divergence between and within *Borrelia* species but not for assignment of *Borrelia* genospecies. We used this approach to analyze some representative southern *Borrelia* strains and to evaluate relationships among them and other genospecies.

MATERIALS AND METHODS

Spirochete isolates and culture conditions. The 20 *Borrelia* strains described in this study were isolated from ticks (*I. scapularis, I. dentatus, I. minor,* or *Dermacentor variabilis*) and animal reservoirs (cotton mouse [*Peromyscus gossypinus*], cotton rat [*Sigmodon hispidus*], and eastern woodrat [*Neotoma floridana*]) from Missouri, Georgia, Florida, South Carolina, and Texas. All analyzed isolates were third passage. Table 1 lists all *Borrelia* strains that were investigated and their origins. The cultures were incubated at 34°C for 1 to 2 weeks (to the stationary phase of growth) and checked under dark-field microscopy to ensure purification, and cells were counted before they were harvested.

Extraction of spirochete DNA. Whole DNA was isolated without separating plasmid and chromosomal DNAs. Briefly, 30 ml of stationary-phase cultures were washed with 0.01 M phosphate-buffered saline (PBS) (pH 7.2) with 5 mM $MgCl_2$ three times at 10,000 rpm (12,096 $\times$ *g*) for 10 min. Washed pellets of the spirochetes were resuspended in 250 μl of TES (50 mM Tris [pH 8.0], 50 mM EDTA, 15% [wt/vol] sucrose). Subsequently, an equal volume of 5 M NaCl as well as 0.01% (vol/vol) sodium deoxycholate were added to the cell suspension. Samples were placed on ice for 30 min. The partially lysed cell suspension was then centrifuged at 10,000 rpm (12,096 $\times$ *g*) for 10 min. After discarding the supernatant fraction, the pellet was resuspended in 250 μl of TES, followed by addition of 250 μl of 10% sodium dodecyl sulfate (SDS) and 3 μl of RNase.

After a 30-min incubation at 37°C, 50 μl of proteinase K (20 mg/ml) was added, and the tubes were incubated at 50°C for 30 min. This solution was subsequently phenol extracted twice, phenol-chloroform-isoamyl alcohol (25:24:1) extracted once, and chloroform-isoamyl alcohol (24:1) extracted once. After the addition of 1/10 (vol/vol) volume of 3.0 M sodium acetate (pH 5.2), 2 volumes of cold ethanol were added to precipitate the nucleic acid. The DNA pellets were resuspended in 50 μl of TE buffer (10 mM Tris [pH 7.6], 1 mM EDTA). DNA samples were stored at −20°C until required for PCR experiments.

Amplification of *ospC* genes. A 627- to 645-bp fragment of *ospC* was amplified by using a pair of primers, *ospC*3 (5′-AAGTGCAGATATTAATGACTTTA-3′) and *ospC*4 (5′-TTTTTTGGACTTTCTGCCACA-3′), based on sequence data in GenBank and previously used primers (38). PCRs were performed in volumes of 50 μl containing 10 mM Tris-HCl (pH 9.0 at 25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 μM dATP, 200 μM dGTP, 200 μM dCTP, 200 μM dTTP, 1.5 U of *Taq* DNA polymerase in storage buffer A (Promega, Madison, Wis.), 1 μM each primer, and 10 ng of extracted DNA. Reactions were performed in a GeneAmp PCR System 9700 (PE Biosystems) for 35 cycles of denaturation at 93°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 1 min, and final extension was carried out at 72°C for 9 min. The PCR products were detected in a 1% agarose gel in 0.5$\times$ Tris-borate-EDTA containing 0.5 μg of ethidium bromide/ml. The gel was photographed with an Eagle Eye II system (Stratagene).

Purification of PCR products, sequence analysis, and phylogenetic analysis. The PCR products were purified by the Wizard PCR prep DNA purification system (Promega). The DNA sequence of *ospC* was determined by using an ABI Prism (model 377). Sequences were aligned manually and by using Clustal W software (28), and phylogenetic trees were constructed by neighbor joining (52), maximum likelihood (22), parsimony methods (62), and unweighted pair group method with arithmetic means analysis (UPGMA) (58) by using phylogenetic analysis using parsimony (PAUP) (62), phylogeny inference package PHYLIP (22), and molecular evolutionary genetics analysis (MEGA) (34). The DNA sequences of *ospC* were translated into amino acid sequences using Protein Machine software, and each deduced amino acid sequence then was aligned using Clustal W as described above. The phylogenetic trees were generated by using PAUP, Clustal W, PHYLIP, and MEGA.

Retrieved sequences. To compare the relationship between our strains and the strains in different genospecies and to conduct phylogenetic analyses, *ospC* sequences of strains from the following genospecies (with strain names and data-

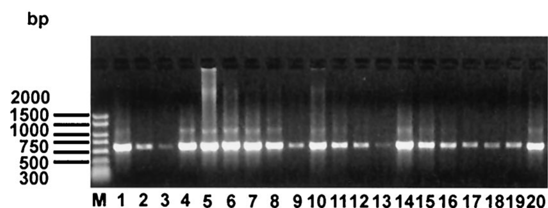

FIG. 1. Amplified *ospC* genes of southern strains of *B. burgdorferi* sensu lato. The PCR products were electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized with a UV light transilluminator. Molecular size standards (in base pairs) are shown to the left of the gel. The 19 southern strains and the reference strain AI-1, FD-1, MI-6, MI-8, MI-9, MOD-1, SCW-30h, SCGT-8a, SCGT-10, SI-10, BC-1, MOS-1b, MOD-5, MOK-3a, SI-1, SM-1, MI-2, MI-5, SCI-2, and B31 are shown in lanes 1 to 20, respectively.

base accession numbers) were used. They included *B. burgdorferi* sensu stricto [X69596 (B31[T]), L81131 (CA4), U91792 (HII), U91799 (IP1), L42887 (IP2), U91797 (IP3), U91798(L5), U91801(P1F), X69589 (PKa), X84783 (TXGW), X84778 (DUN), X84765 (BUR), L42868 (ZS7), X81522 (PBre), X73625 (DK7), U91795 (61BV3), U91794 (35B808), U04281 (HB19), L42894 (Z8691), L42893 (297), U91790 (212), U91802 (MIL), X81524 (T255), L42899 (21347), U01892 (2591), U04240 (N40), L42894 (28691), L42895 (28354), X84779 (MUL), X84782 (KIPP), X84785 (272), L42897 (26815), L81130 (SON188), L42896 (27579), X83555 (*Borrelia pacificus*), U91793 (Z136), U91796 (20006), U91800 (NE56), U91791 (ESP-1), L25413 (CA11.2), AF029860 (OC1), AF029861 (OC2), AF029862 (OC3), AF029863 (OC4), AF029864 (OC5), AF029866 (OC7), AF029865 (OC6), AF029867 (OC8), AF029868 (OC9), AF029869 (OC10), AF029870 (OC11), AF029871 (OC12), AF029872 (OC13), AF065143 (OC16), and AF065144 (OC17)], *B. afzelii* [L42871 (VS461[T]), X84777 (DK9), L42892 (ACAI), X84775 (DK5), L42874 (Orth), L42884 (J1), X81523 (P1J7), L42888 (H9), L42873 (Simon), L42890 (E61), X84768 (DK15), X84774 (DK4), X84776 (DK8), X73624 (DK26), X84767 (DK21), L42883 (JSB), X81521 (PBo), X84771 (DK3), X84766 (DK2), X73627 (DK1), X84769 (DK22), X83552 (PLud), X62162 (PKo), X69590 (PWudI), X80255 (PLe), D49501 (HT10), D49502 (HT25), and D49503 (HT61)], *B. garinii* [X69595 (PBi), X73626 (DK6), X83554 (PTrob), L42800 (KL11), L42869 (W), X83553 (PHei), X81526 (WAB-Sou), X84770 (DK29), X73623 (DK27), X83556 (N34), L42879 (M57), X81525 (Tis1), L42889 (H13), L42900 (20047[T]), L42878 (NBS16), L42870 (VSDA), L42875 (NBS23), L42863 (153), X84773 (DK35), L42891 (BITS), L42882 (KL10), L42877 (NBSlab), L42886 (IP90), X69594 (pBr), X69593 (TN), X69592 (T25), D49499 (HT17), D49500 (JEM4), D49377 (HT57), and D49378 (HT64)], *B. bissettii* [U04280 (DN127[T]) and U04282 (25015)], *B. andersonii* [L42866 (21123[T]) and L42864 (19857)], *B. valaisiana* [L42872 (VS116[T])], and *Borrelia* spp. [D49504 (HT22), D49505 (JEM1), D49506 (JEM2), D49381 (HT37), D49507 (HT19), D49508 (JEM3), and D49509 (HT55)].

Nucleotide sequence accession numbers. The *ospC* nucleotide sequences of 19 *B. burgdorferi* sensu lato isolates were deposited in the GenBank database, and their accession numbers are AF278582 (MI-2), AF278583 (SCW-30h), AF278584 (MI-9), AF278585 (MOK-3a), AF278586 (MOD-5), AF278587 (BC-1), AF278588 (SCGT-8a), AF278589 (SCGT-10), AF278590 (MI-8), AF278591 (AI-1), AF278592 (FD-1), AF278593 (SI-1), AF278594 (SM-1), AF278595 (SCI-2), AF278596 (MI-6), AF278597 (MOD-1), AF278598 (MOS-1b), AF278599 (SI-10), and AF278600 (MI-5).

RESULTS

Amplification and sequencing of *ospC* genes. Excluding TXW-1, 606- to 624-bp coding regions of the *ospC* genes of the 19 southern *B. burgdorferi* sensu lato strains were amplified (Fig. 1) and sequenced. There was no suggestion of a relationship between the lengths of the *ospC* genes and the spirochete genospecies. However, the longest genes (624 bp) of *ospC* were found in SCGT-8a, SCGT-10, MI-6, and MOD-1 and the shortest one in BC-1 (606 bp); most southern strains have an average *ospC* gene length of 609 to 621 bp. Although various PCR conditions were used to amplify the *ospC* gene in strain TXW-1, failure suggests that TXW-1 may lack this gene or that the undetected gene in this isolate is truncated or otherwise altered at the primer sites. To confirm our sequencing results, the *ospC* gene of reference strain B31 was sequenced and compared to the B31 sequence retrieved from GenBank; identical results were obtained.

Analysis of *ospC* sequences of southern strains. A similarity matrix of OspC amino acid sequences indicated a high degree of heterogeneity, and amino acid identities ranged from 59.9 to 100%; OspC from most strains ranged from 60 to 80% identity (Table 2). The lowest degree of similarity (59.9%) was found between SM-1 (*B. burgdorferi* sensu stricto) and 20047 (*B. garinii*). The highest degrees of similarity were shown between strains of *B. burgdorferi* sensu stricto, i.e., SM-1 and SI-1 or SCI-2 (99.5 and 100%), MI-2 and B31 (99.5%), *B. andersonii* strains MOS-1b and SI-10 (99.5%), *B. bissettii* strain MI-9, and *B. burgdorferi* sensu stricto strain MI-5 (99.5%), *B. andersonii* strain MOD-1 and *B. bissettii* strain MI-6 (99%), *B. bissettii* strains SCGT-8a and SCGT-10 (98.1%), MI-9 and FD-1 (95.6%), and *B. burgdorferi* sensu stricto strain MI-5 and *B. bissettii* strain FD-1 (95.1%). As shown above, high degrees of similarity among strains were usually found in those from the same genospecies but also sometimes among strains in different genospecies (Table 2), for example, between *B. burgdorferi* sensu stricto and *B. bissettii* or between *B. andersonii* and *B. bissettii*. Results indicated that *B. burgdorferi* sensu stricto, *B. bissettii*, and *B. andersonii* from North America are closely related.

The amino acid sequences of species-specific epitopes of southern strains varied between positions 28 and 31. Most southern *B. bissettii* and *B. andersonii* strains have an S at position 28, but southern *B. burgdorferi* sensu stricto strains either have an A or lack an amino acid at this position. At positions 29 and 30, most southern *B. andersonii* and *B. bissettii* strains have A and S, but southern *B. burgdorferi* sensu stricto strains have T and S or S and V. At position 31, southern *B. burgdorferi* sensu stricto strains have either an A or a gap; however, most southern *B. bissettii* and *B. andersonii* strains have T, and three *B. andersonii* strains have N.

Except for this region, each of the strains MOD-5, MOK-3a, SI-1, SM-1, SCI-2, SCW-30h, and AI-1 has a gap at position 70. Each of the strains SCGT-8a and SCGT-10 has an insertion of T at position 88. Strain BC-1 has a deletion at position 79, and strains MI-6 and MOD-1 have a 3-amino-acid insertion, G, L, and N. Strains BC-1, B31, MI-2, 25015, MI-5, MI-9, FD-1, MOD-5, and MOK-3a each have a deletion at position 115. Strains SCGT-8a and SCGT-10 have an insertion of N and G and strains SI-10 and MOS-1b have an insertion of D and G at positions 146 and 147. These differences in OspC sequences among southern strains reflect significant diversity at the genospecies level.

An alignment of the deduced amino acid sequences indicated that both the C-terminal and N-terminal ends of OspC appear to be conserved. The amino acid sequence variation was observed in the internal part of this protein; a variable region was located from amino acids 72 to 191 among these southern strains. Two hypervariable regions were located between amino acids 84 and 96 and between amino acids 146 and 176. In comparison with the previously reported hypervariable regions in other strains (30), the first hypervariable region

among southern strains was located between amino acids 84 and 96 rather than between amino acids 83 and 95. The second hypervariable region among southern strains was located between amino acids 146 and 164 and extended to amino acid 176 rather than between amino acids 143 and 162. There were three identical amino acids in the second hypervariable region.

There are four domains in the OspC amino acid sequence according to a previous report (38): the conserved amino-terminal domain (C1), two variable domains (V1 and V2), and the semivariable domain (SV). For southern strains, C1 is located from amino acids 35 to 86, V1 from amino acids 86 to 99, SV from amino acids 99 to 118, and V2 from amino acids 118 to 191. The locations of these domains are shown in Table 3.

Lateral transfer and internal gene recombination of *ospC* gene and OspC sequence diversity. A close identity was found between *B. burgdorferi* sensu stricto and *B. bissettii* and between *B. bissettii* and *B. andersonii* (Tables 2 and 3). The close identity (indicated in Table 3) among some amino acid sequences of southern strains and other strains belonging to different *B. burgdorferi* genospecies suggests that a lateral transfer and internal gene recombination of the *ospC* gene may have occurred among the three genospecies. Both phylogenetic trees also suggest that lateral transfer and internal gene recombination of the *ospC* gene occurred among these three genospecies (Fig. 2 and 3). Based on the result in which MI-6 (*B. bissettii*) was misplaced in the SI-10 cluster (*B. andersonii*) in the phylogenetic tree (Fig. 2), *B. andersonii* appears to be the donor and *B. bissettii* the recipient. However, *B. andersonii* might have obtained part of the *ospC* sequence from *B. bissettii* based on the grouping in which MOD-5 (*B. andersonii*) was misplaced in the FD-1 group (*B. bissettii*). Genetic transfer could happen in both directions. Moreover, B31 and MI-2 were misplaced in the 25015 group (*B. bissettii*), suggesting that *B. burgdorferi* sensu stricto might have obtained part of the *ospC* gene from *B. bissettii*.

Clinical and epidemiological implication of *ospC* groups. Each *ospC* type is a group of strains in which divergence is greater than 8% between groups and less than 1% within groups (27, 57, 64). A branch length of 0.04 corresponds to 8% divergence between two sequences and is the cutoff that defines the *ospC* group (9, 21). Based on this definition, the 19 southern strains can be divided into 12 *ospC* groups (except for the MI-5, MI-9, and FD-1 group, whose branch length is 0.037 and is close to the cutoff) (Fig. 2, Table 4). Of the 12 southern strain *ospC* groups, two groups belong to the *B. burgdorferi* sensu stricto genospecies. One is the A group (Table 4) and contains MI-2 along with the IP1 group, all of which belong to a so-called invasive cluster (9, 57). MI-2 most closely aligns with a group that possesses proven human invasive strains.

Transmission experiments show that this group of southern spirochetes are able to infect mice and hamsters and that the typical vector of Lyme disease, *I. scapularis*, can acquire the spirochetes from infected mammals (J. H. Oliver et al., unpublished data). Another *B. burgdorferi* sensu stricto group (V group) contains three southern strains, SI-1, SM-1, and SCI-2, and is a typical southern *B. burgdorferi* sensu stricto group. MI-5 (*B. burgdorferi* sensu stricto) and MI-9 and FD-1 (*B. bissettii*) comprise a mixed group, Y. MI-8, the undescribed new genospecies, constitutes the Z group. There are three *B.*

TABLE 2. Amino acid similarity between OspC sequences from different *B. burgdorferi* sensu lato isolates

Isolate	% Amino acid similarity with OspC sequence from isolate:																								
	DN127	VS116	21123	25015	VS416	20047	B31	SI-1	SI10	SM1	SCI2	SCW30h	AI1	FD-1	MI-2	MI-5	MI-6	MI-8	MI-9	SCGT-8a	SCGT-10	MOD-1	MOD-5	BC-1	MOK-3a	MOS-1b
DN127	100.0	67.3	69.8	69.1	69.3	69.3	68.0	67.0	70.2	66.5	67.0	73.0	70.7	75.0	67.5	73.0	70.2	73.1	72.6	75.1	76.6	71.2	70.0	69.3	75.9	69.8
VS116		100.0	66.8	66.3	69.8	63.4	68.8	63.9	64.9	63.3	63.9	63.3	68.3	69.3	68.3	67.3	70.3	69.3	66.8	66.8	68.8	70.8	62.4	62.9	65.3	64.4
21123			100.0	67.6	63.9	62.9	66.5	70.0	81.6	66.5	70.0	65.0	71.2	69.6	66.5	70.6	73.9	70.7	70.1	70.5	71.0	74.4	67.0	72.3	68.5	82.1
25015				100.0	70.8	64.4	82.8	69.5	68.6	69.0	69.5	71.4	73.5	81.4	82.3	80.4	75.0	75.5	80.0	74.0	73.5	75.0	77.8	68.8	79.8	68.1
VS461					100.0	74.8	73.8	67.8	63.9	67.3	67.8	70.8	70.8	70.8	73.3	69.8	69.3	70.3	69.3	69.8	69.8	69.3	67.8	65.3	68.3	63.4
20047						100.0	64.4	60.4	63.9	59.9	60.4	66.8	63.4	66.8	64.9	64.9	63.9	68.3	64.4	69.8	68.8	63.9	62.9	60.4	63.9	63.4
B31							100.0	69.0	65.0	68.5	69.0	72.4	70.0	77.3	99.5	76.4	71.9	73.4	75.9	68.5	67.5	71.4	72.4	66.3	74.4	64.5
SI-1								100.0	67.5	99.5	100.0	70.9	72.4	71.4	68.5	68.5	68.5	67.0	69.0	64.0	64.5	68.5	69.5	65.8	70.9	67.0
SI10									100.0	67.0	67.5	66.5	70.2	69.6	65.0	69.9	72.9	70.7	69.1	69.6	70.5	73.4	65.5	71.3	69.5	99.5
SM1										100.0	99.5	70.4	71.9	70.9	68.0	68.0	68.0	66.5	68.5	63.5	64.0	68.0	69.0	65.3	70.4	66.5
SCI2											100.0	70.9	72.4	71.4	68.5	68.5	68.5	67.0	69.0	64.0	64.5	68.5	69.5	65.8	70.9	67.0
SCW-30h												100.0	77.3	72.4	71.9	71.4	69.0	73.4	70.9	70.0	69.5	68.5	68.0	64.9	71.9	66.0
AI-1													100.0	76.5	69.5	73.5	75.1	75.1	73.0	68.8	70.2	74.6	71.4	68.3	76.4	69.8
FD-1														100.0	76.8	95.1	77.9	80.4	95.6	75.0	76.5	77.9	82.8	73.3	81.8	69.1
MI-2															100.0	75.9	71.4	72.9	75.4	68.0	67.0	70.9	71.9	66.3	74.0	64.5
MI-5																100.0	76.5	79.4	99.5	72.1	73.5	76.0	81.8	74.3	80.3	69.1
MI-6																	100.0	75.6	76.0	73.6	74.5	99.0	70.4	73.3	73.4	72.5
MI-8																		100.0	78.9	75.1	77.1	75.6	71.4	68.8	73.9	70.2
MI-9																			100.0	72.5	74.0	75.5	82.3	73.8	79.8	68.6
SCGT-8a																				100.0	98.1	73.6	68.5	67.8	70.0	69.6
SCGT-10																					100.0	74.5	70.4	67.8	70.9	70.5
MOD-1																						100.0	70.0	73.3	74.4	72.9
MOD-5																							100.0	69.8	78.8	65.5
BC-1																								100.0	71.3	70.8
MOK-3a																									100.0	69.0
MOS-1b																										100.0

TABLE 3. Amino acid sequences of OspC proteins of southern *B. burgdorferi* sensu lato and other strains deduced from the nucleotide sequences of *ospC*s aligned with Clustal W[a]

Isolate	Species specific sequences ◄► C1		V1 ◄► SV ◄►		V2			
SCGT–8a	SAILMTLFLFISCNNSGKDGNSAST-NPADESVKGPNLTEISKKIMDSNTVVLAVKEVEA	59	LLSSIDELATKAIGKKIDANGSLVADATDFNTSLLAGAYAISSLITEKLN---KLKNSEG	116	LKEKIAEAKKCSDDFTKKLKDSHQELGVANGAATADNAKKAILKTNATKDKGAEELEKLF	176	KAVESLSKAAQDMLTNSVKELTSPVVGKSPKX	208
SCGT–10	SAILMTLFLFISCNNSGKDGNSAST-NPADESAKGPNLTEISKKITDSNTVVLAVKEVET	59	LLASIDELATKAIGKKIDANGSLVADATDFNTSLLAGAYAISSLITEKLN---KLKNSEG	116	LKEKIAEAKKCSDDFTKKLKDSHQELGVANGAATADNAKKAILKTNATKDKGAEELEKLF	176	KAVESLSKAAQDMLTNSVKELTSPVVGKSPKX	208
DN127	SAILMTLFLFISCNNSGKDGNSAST-NPADESAKGPNLTEISKKITDSNAIVLAVKEVET	59	LLLSIDELA-KAIGKKINNNG--LDVLQNFNASLLGGAHTISKLITEKLS---KLNGSEE	113	LKEKIEAAKKCSDDFTKKLQSSHAELGVAGGATTDENAKKAILKSNADKTKGADELGKLF	173	ESVESLAKAAKEMLANSVKELTSPVVAETPKX	205
SI–10	SAILMTLFLFISCNNSGKDGNSAST-NPVDESVKGPNLIEISKKITDSNAVVLAVQEVET	59	LLASIDEIAKKAIGQRIAPGNTLTADA-SRNGSLIVGVYTISTLITGKLN---GLKDLED	115	LKEKIEKAKKCSTEFTKKLLDSHAELGQADGAVTDDNAKRAILKTHNNTDKGAKELKDLS	175	ESVESLAKAAKEMLNNAVKELTKPVVAEAPKX	207
MOS–1b	SAILMTLFLFISCNNSGKDGNSAST-NPVDESVKGPNLIEISKKITDSNAVVLAVQEVET	59	LLASIDEIAKKAIGQRIAPGNTLTADA-SRNGSLIVGVYTISTLITGKLN---GLKDLED	115	LKEKIEKAKKCSTEFTKKLLDSHAELGQADGAVTDDNAKRAILKTHNNTDKGAKELKDLS	175	ESVESLAKAAKEMLNNAVKELTKPVVTEAPKX	207
21123	SAILMTLFLFISCNNSGKDGNSASN-NSADESVKGPNLVEISKKITDSNAVVLAVQEVET	59	LLASIDELAKKAIGQRIDQGNKLAVDA-DHNGSLLAGVYTISTLITEKLN---SLKISEE	115	LKEKIEKAKKYSTEFTKKLSDSHGVLGQVGGNATDVNAKAAILKTNQTTDKGAKELKELS	175	ESVESLAKAAKEMLNDSIKELTKPVVTEAPKX	207
BC–1	SAILMTLFLFISCNNSGKDGNSASN-NSADESVKGPNLIEISKKITDSNVVVLAVKEIET	59	LLSSIDELGNKAIGKLIS-ANGLNVQA-GQNGSLLAGAHAISSLITQKLS---ALN-SEE	113	LKEKIKDAKDCSEKFTKKLSESHADLGQN---ATDDNAKRAILKTHTDTDKGAKELKELS	170	ASVESLAKAAKELLNNAVKELTKPIVAESPKX	202
MI–6	SAILMTLFLFISCNNSGKDGNSAST-NPVDESVKGPNLTEISKKITDSNAVVLAVKEVET	59	LLASIDQLATKAIGKKIDQNNALGTQA-GQNGSLLAGAYTISTLITEKLSGLNALKNAEE	118	LKEKIKDAKKFSEDFTKKLSDNHADLSPA--AATDDNAKAAILKTNNTKDKGAKELKELS	176	ESVEALSKAAQAMLTNSVKELTSPVVAESPKX	208
MOD–1	SAILMTLFLFISCNNSGKDGNSAST-NPVDESVKGPNLTEISKKITDSNAVVLAVKEVET	59	LLASIDQLATKAIGKKIDQNNALGTQA-GQNGSLLAGAYTISTLITEKLSGLNALKNAEE	118	LKEKIKDAKKFSEDFTKKLSDSHADLSPA--AATDDNAKAAILKTNNTKDKGAKELKELS	176	ESVEALSKAAQAMLTNSVKELTSPVVAETPKX	208
831	SAILMTLFLFISCNNSGKDGN-TSA-NSADESVKGPNLTEISKKITDSNAVLLAVKEVEA	58	LLSSIDEIAAKAIGKKIHQNNGLDTEN-NHNGSLLAGAYAISTLIKQKLD---GLK-NEG	113	LKEKIDAAKKCSETFTNKLKEKHTDLGKE--GVTDADAKEAILKTNGTKTKGAEELGKLF	171	ESVEVLSKAAKEMLANSVKELTSPVVAESPKX	203
MI–2	SAILMTLFLFISCNNSGKDGN-TSA-NSADESVKGPNLTEISKKITDSNAVLLAVKEVEA	58	LLSSIDEIAAKAIGKKIHQNNGLDTEN-NHNGSLLAGAYAISTLIKQKLD---GLK-NEG	113	LKEKIDAAKKCSETFTNKLKEKHTDLGKE--GVTDADAKEAILKTNGTKTKGAEELGKLF	171	ESVEVLSKAAKEMLANSVKELTNPVVAESPKX	203
25015	SAILMTLFLFISCNNSGKDGNAAST-NPADESVKGPNLTEISKKITDSNAVVLAVKEVGA	59	LLTSIDELATKAIGKKIHQNNGLDTEN-NHNGSLLAGAYAISTLITQKLG---GLK-NEE	114	LKEKIAAVKKCSEEFTNKLKSSHTELGKQ--DAQDDDAKKAILRTHNTKDKGAEELDKLF	172	KAVENLSKAAKEMLSNSVKELTSPVVAESPKX	204
MI–5	SAILMTLFLFISCNNSGKDGNSAST-NPADESTKGPNLTEISKKITDSNAVVLAVKEVET	59	LIASIDELADKAIGKKIQQNNGLGTEN-NHNGSLLAGVYAISTLITQKLS---SLN-SEE	114	LKEKIKEAKDCSEKFTKKLETGHAELGKE--DATDDNAKKAVLKTNADKSKGAEELEKLF	172	KSVESLAKAAKESLNKSVKELTSPVVAESPKX	204
MI–9	SAILMTLFLFISCNNSGKDGNSAST-NPADESTKGPNLTEISKKITDSNAVVLAVKEVET	59	LIASIDELADKAIGKKIQQNNGLGTEN-NHNGSLLAGVYAISTLITQKLS---SLN-SEE	114	LKEKIKEAKDCSEKFTKKLETGHAELGKE--DATDDNAKKAVLKTNADKSKGAEELEKLF	172	KSVESLAKAAKESLNKSVKELTSPVVAKSPKX	204
FD–1	SAILMTLFLFISCNNSGKVGNSAST-NPADESTKGPNLTEISKKITDSNAVVLAVKEVET	59	LIASIDELADKAIGKKIQQNNGLGTEN-NHNGSLLAGAYAISTLITQKLS---KLN-SEE	114	LKEKIKEAKKCSEKFTKKLETGHAELGKE--DATDDNAKKAILKTNADKSKGAEELEKLF	172	KSVESLAKAAKEMLTNSVKELTSPVVAKNPKX	204
MOD–5	SAILMTLFLFISCNNSGKDGNSASN-NSADESAKGPNLIEISKKITDSNAVVLAVKEVET	59	LIASIDELA-KAIGKKIQQNNGLGTEN-NQNGSLLAGVHAISTLITEKLN---ALN-SET	113	LKEKIKEVKKCSENFTNTLKQNHTELGKK--DANDDDAKKAILRTNGDKTKGAKELEDLF	171	KAVESLAKAAKEILNNSVKELTSPVVGKNPKX	203
MOK–3a	SAILMTLFLFISCNNSGKDGNSAST-NPADESVKGPNLTEISKKITDSNAVVLAVKEVET	59	LIASIDELA-KAIGKKIEPTGGLGTDN-NHNGSLLSGAHAISTLITQKLS---ALN-SEE	113	LKGKIEDVKKCSQEFTNQLKNSHTELGKQ--DATDENAKKAILKTNAAKDKGAEELGELF	171	ESVESLSKAAKEMLANAVKELTSPVVAETPKX	203
SI–1	SAILMTLFLFISCNNSGKDGNASV--NSADESVKGPNLVEISKKITDSNAVVIAVKEVET	58	LLVSIDELA-KAIGKKIEAGGTLGSDG-AHNGSLLAGAYKIATEITANLS---KLKASED	113	LKEKITKAKECSEKFTDKLKSENAALGKQ--DASDDDAKKAILKTHNDITKGAKELKELS	171	ESVETLLKAAKEMLANSVKELTSPVVAKNPKX	203
SM–1	SAILMTLFLLISCNNSGKDGNASV--NSADESVKGPNLVEISKKITDSNAVVIAVKEVET	58	LLVSIDELA-KAIGKKIEAGGTLGSDG-AHNGSLLAGAYKIATEITANLS---KLKASED	113	LKEKITKAKECSEKFTDKLKSENAALGKQ--DASDDDAKKAILKTHNDITKGAKELKELS	171	ESVETLLKAAKEMLANSVKELTSPVVAKNPKX	203
SCI–2	SAILMTLFLFISCNNSGKDGNASV--NSADESVKGPNLVEISKKITDSNAVVIAVKEVET	58	LLVSIDELA-KAIGKKIEAGGTLGSDG-AHNGSLLAGAYKIATEITANLS---KLKASED	113	LKEKITKAKECSEKFTDLKSENAALGKQ--DASDDDAKKAILKTHNDITKGAKELKELS	171	ESVETLLKAAKEMLANSVKELTSPVVAKNPKX	203
SCW–30h	SAILMTLFLFISCNNSGKDGNSAS--NTADESVKGPNLTEISKKIADSNAVVLAVKEVEA	58	LLASIDELA-KAIGKKIKNDGSLEAEA-NRNESLVAGAYTISALITQKLG---KLKNSEE	113	LKEKISNAKDCAEAFTKKLKDNQAELGVQ--GVTDENSKKAILKTHGDKTKGAEELGKLF	171	ESVGNLSKAAKELLDNSVKELTSPVVAENPKX	203
AI–1	SAILMTLFLFISCNNSGKDGNSASTNNPADESTKGPNLTEISKKITDSNAVVLAVKEVVT	60	LIASIDELS-KAIGKKFNQNGGLDAEA-DHNGSLLSGAYTISTLITQKLG---KLKNSEE	115	LKEKIENAKKCSEEFTKKLKDNQADLGKK--DATDENAKKAILKTHGTTDKGAKELKELS	173	ESVEILIKAAKDMLTNSVKALTSPVVAENPKX	205
MI–8	SAILMTLFLFISCNNSGKDGNSAST-NPADESAKGPNLTEISKKITDSNTVVLAVKEVET	59	LLASIDELATKAIGKKIQQNGGLAAEA-GHNGTLLAGAYTISKLITQKLD---GLKNTEK	115	LKEIENAKKCSEDFTKKLEGEHVVLGIE--NVTDENAKKAILITDAAKDKGAAELEKLF	173	KSVESLAKAAQESLANSVKELTSPVVAENPKX	205
VS461	SAILMTLFLFISCNNSGKGGDIAST-NP-DESAKGPNLTEISKKITDSNAVVLAVKEVEA	58	LLSSIDELA-KTIGKKIEANG-LGNEA-DKNGSLLAGAYAISTLIKQKLD---GLKGLEG	112	LNKEIAEAKKCSEAFTKKLQDSNADLGKHN--ATDADSKEAILKTNGTKTKGAKELEELF	170	KSVESLSKAAKEALSNSVKELTSPVVAESPKX	202
20047	SAILMTLFLFISCNNS--GGDTAST-NP-DESVKGPNLTEISKKITDSNAFVLAVKEVEA	56	LISSIDELA-KAIGQRIQQNG-LVADA-GHNSALLAGAHEISILITQKLD---GLKGLEG	110	LKAEIAEAKKYSEAFTKKLKDNHAQLGIQNGASLDDEAKKAILKTNVDKTKGAEELEKLF	170	KSVESLSKAAQEALTNSVKELTNPVVAETPKX	202
VS116	SAILMTLFLFISCNNS--GGDTAST-NPVDESAKGPNLTEISKKITDSNAIVLAVKEVET	57	LLASINEIANKGIGKKINQNG-LDNLT-DHNGSLIAGAYVISTLITEKLN---NLKNSEG	112	LKEKIKKVKECSDKFTKKLTTSNGDLGKEN--VTDAHAQAAILKTNPTNDKGAKELGELF	170	ESVEILSKAAQEALTNSIAELTSPVVAENPKX	202
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[a] Dashes indicate gaps. The conserved amino-terminal (C1), variable (V1 and V2), and semivariable (SV) domains and species-specific motif of the OspC proteins are indicated. Identical amino acid sequences (indicated by asterisks) of strains suggest a lateral genetic transfer of part of the *ospC* genes within and between genospecies.

bissettii groups, AI-1 (A), SCW-30h (B1), and the SCGT-8a–SCGT-10 group (C1), and two mixed groups, the D1 group (MI-6 and MOD-1) and the Y group (FD-1, MI-9, and MI-5). The *B. andersonii* genospecies includes BC-1 (F1), the MOS-1b–SI-10 group (G1), MOD-5 (H1), and MOK-3a (I1). Except for the mixed groups, which comprise different genospecies, the neighbor-joining tree grouped MOK-3a with DN127, and the MI-2 group clustered with 25015 (Fig. 2). It appears that horizontal genetic transfer and internal gene recombination of the *ospC* gene occurred between *B. burgdorferi* sensu stricto and *B. bissettii* and between *B. bissettii* and *B. andersonii*.

Except for the MI-2 strain, which is included in the invasive cluster (*ospC* group A), most southern strains formed separate groups (Fig. 3, Table 4). We identified them as major *ospC* groups including V to I1. Of the 12 southern major *ospC* groups, two were *B. burgdorferi* sensu stricto groups, three were *B. bissettii* groups, four were *B. andersonii* groups, one was an undescribed genospecies group, and two were mixed groups (formed by *B. burgdorferi* sensu stricto and *B. bissettii* and by *B. bissettii* and *B. andersonii*) (Fig. 3, Table 4).

Phylogenetic analysis of *ospC* gene. To compare and further investigate the phylogenetic relationships within and among southern strains and other genospecies available in GenBank, phylogenetic trees were constructed based on the almost complete amino acid sequences of OspC and partial nucleotide sequences of *ospC*. Comparison of phylogenetic trees based on these results indicated that all trees were consistent, with minor variation (Fig. 2 and 3).

Phylogenetic trees inferred from 606 to 624 bp of nucleotide sequences of *ospC* (from nucleotides 18 to 643) and 202 to 208 corresponding amino acids of OspC (from amino acids 7 to 218) (Table 3) of southern strains and reference strains of *B. burgdorferi* sensu stricto, *B. andersonii*, *B. bissettii*, *B. garinii*, *B. afzelii*, and *B. valaisiana* were exactly the same. There were four major clusters in the neighbor-joining tree (Fig. 2). Clearly, genetic diversity exists among southern strains, especially *B. bissettii* and *B. andersonii*, compared to United States *B. burgdorferi* sensu stricto strains. *B. bissettii* strains were present in six branches; in five of them, *B. bissettii* combined with *B. andersonii*, *B. burgdorferi* sensu stricto, and the undescribed genospecies MI-8. Also, diverse *B. andersonii* strains were present in three branches.

The deduced OspC amino acid sequences of southern strains were aligned and compared with previously published OspC sequences of other genospecies (Table 3). Significant variability was observed among the OspC sequences present in the 19 southern *B. burgdorferi* sensu lato strains and other genospecies. According to a previous study of the OspC sequence and its polymorphism in *B. burgdorferi* (69) and the N-terminal sequences of Vmp3 and Vmp33 of *B. hermsii* (12, 16), a high degree of variation exists between the Vmps of *B. hermsii* and OspCs of *B. burgdorferi* sensu lato. Species-specific epitopes were found between amino acids 20 and 33, which is highly conserved among *B. afzelii* and *B. garinii* strains but is significantly different compared with that of *B. burgdorferi* sensu stricto strains. The highest degree of genetic diversity among them was observed in the two variable domains (V1 and V2), the semivariable domain (SV), and the species-specific epitopes (between amino acids 28 and 31). Except for these regions, amino acid insertion, deletion, and replacement of OspC among the three genospecies occurred at many positions in the variable regions. These differences in OspC sequences

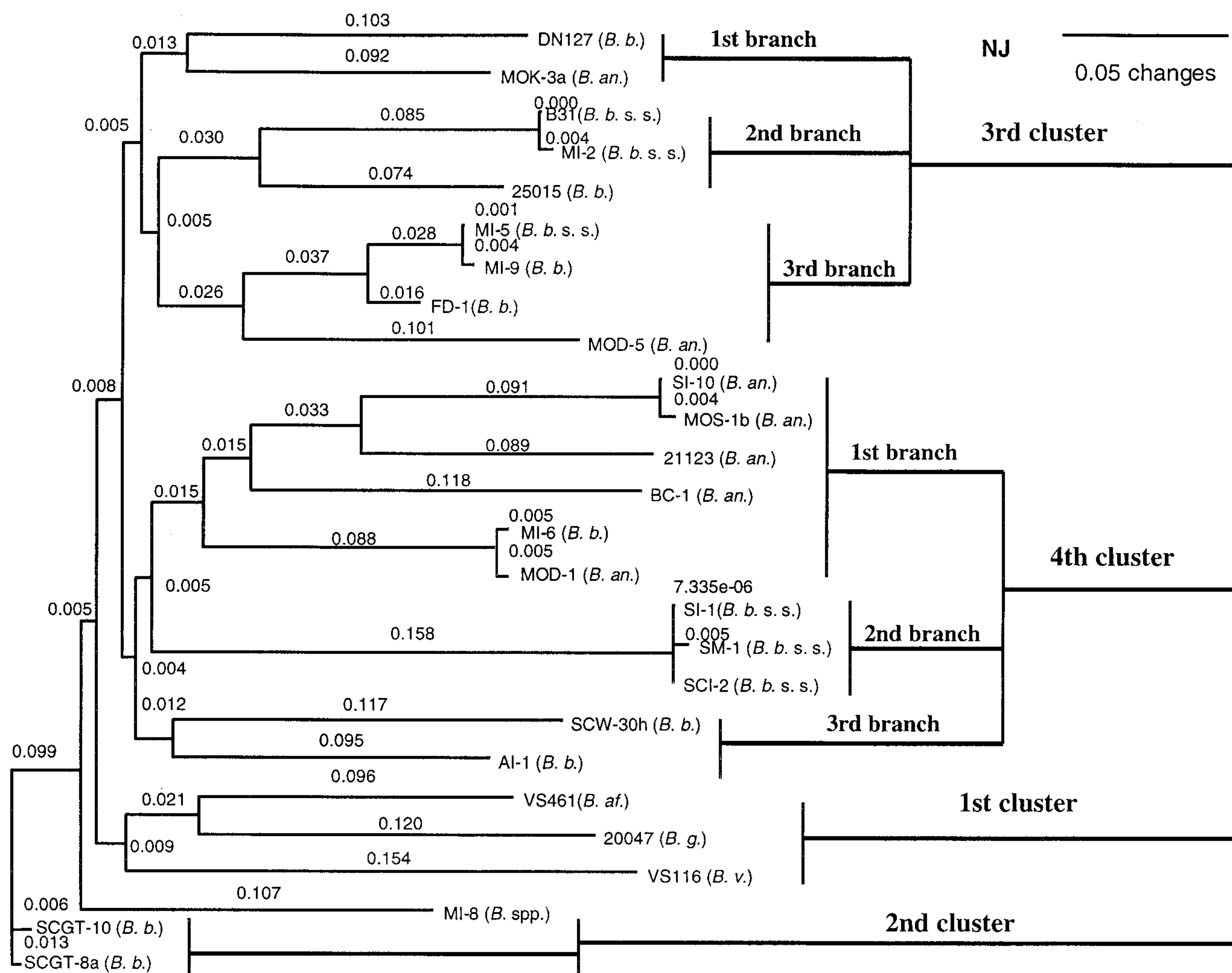

FIG. 2. Phylogenetic tree derived from the deduced amino acid sequences of southern and reference strains of *Borrelia burgdorferi* sensu lato. The tree was constructed with PAUP software and was based on a comparison of 208 OspC amino acids. *B. b.* ss, *Borrelia burgdorferi* sensu stricto; *B. b.*, *Borrelia bissettii*; *B. an.*, *Borrelia andersonii*; *B. g.*, *Borrelia garinii*; *B. af.*, *Borrelia afzelii*; *B. v.*, *Borrelia valaisiana*.

among southern strains reflect the diversity at the strain and genospecies levels.

Phylogenetic trees were also derived from 344 bp of nucleotide sequences (from nucleotides 108 to 552) of *ospC* genes and 146 deduced amino acid sequences (from amino acids 36 to 184) of OspCs. This fragment included the variable regions of *ospC*. The southern strains were compared with some other strains available in GenBank, and two trees indicated similar results; one is presented in Fig. 3. There were 17 clusters in the UPGMA tree which could be divided into two parts. The first part consisted of *B. burgdorferi* sensu stricto (including both North American and some European strains), *B. andersonii*, and *B. bissettii* strains. The second part consisted of Eurasian *B. garinii*, *B. afzelii*, and some European *B. burgdorferi* sensu stricto strains. The *B. burgdorferi* sensu stricto strains separated into 11 clusters, 8 of them located in the first part of the tree; in fact, they were the major strains in that part. Three of them shared clusters with *B. garinii* and *B. afzelii* in the second part of the tree. Three strains in three clusters of the second part were European *B. burgdorferi* sensu stricto strains (20006, Z136, ESP1, and NE56); no North American strains were found in that part.

Compared to European *B. burgdorferi* sensu stricto strains, North American *B. burgdorferi* sensu stricto strains appear to have greater *ospC* heterogeneity. North American *B. burgdorferi* sensu stricto strains were dispersed in all eight clusters in the *B. burgdorferi* sensu stricto part of the tree, and three clusters were exclusively North American strains, but European *B. burgdorferi* sensu stricto strains were included in only five clusters (Fig. 3). Several of the European *B. burgdorferi* sensu stricto strains were closely related to North American *B. burgdorferi* sensu stricto strains, inasmuch as they shared five clusters. Nevertheless, they were different. European *B. burgdorferi* sensu stricto strains appeared genetically closer to each other and grouped into two major clusters. For example, PKa, P1F, IP1, IP2, IP3, and HII were located in the same branch as strains 35B808, 61BV3, DK7, PBre, and ZS7. Interestingly, only European *B. burgdorferi* sensu stricto strains were found in the European genospecies *B. garinii* and *B. afzelii* part of the tree (Fig. 3).

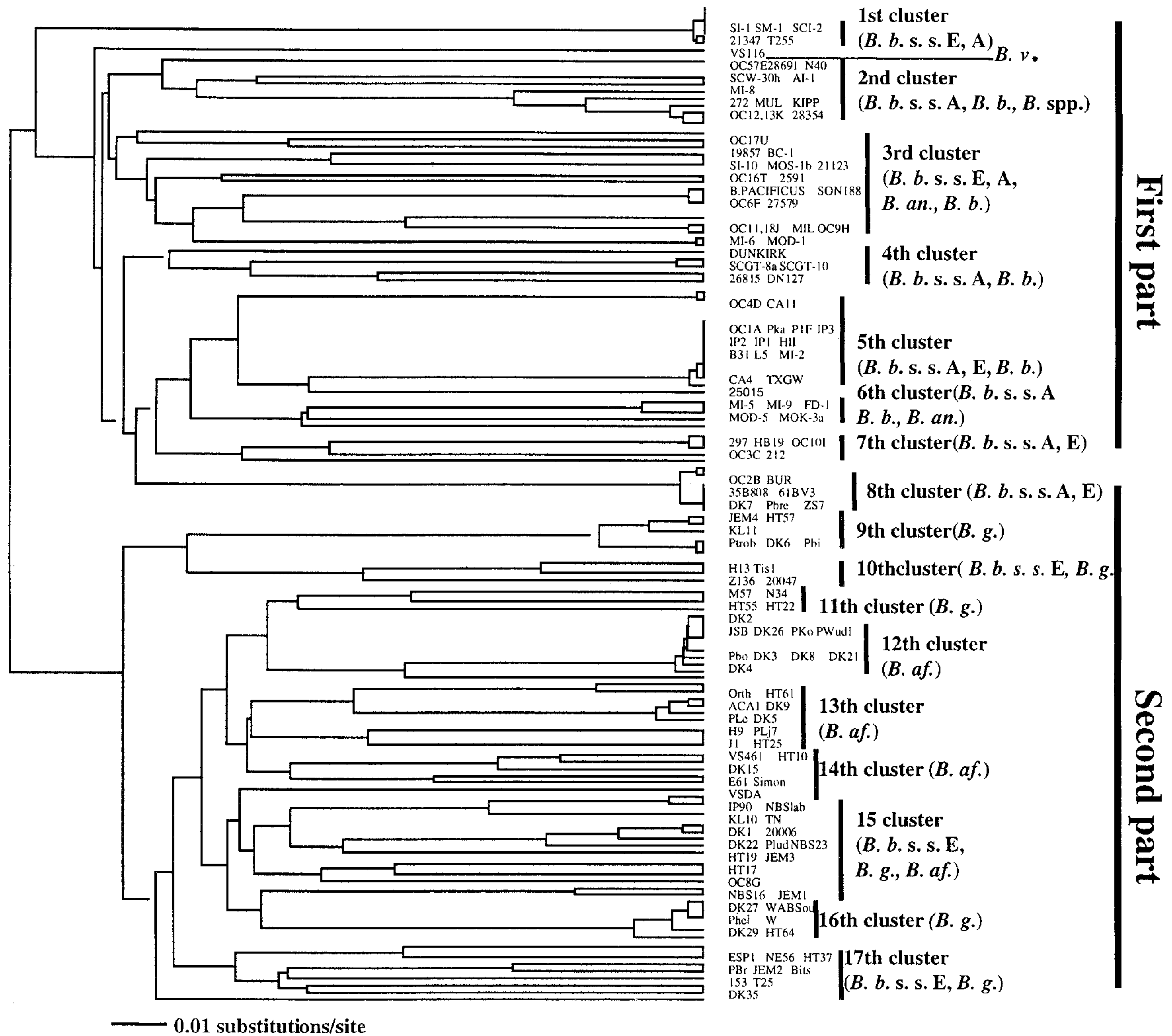

FIG. 3. Phylogenetic tree determined from the DNA sequence of the *ospC* genes of southern and other genospecies strains of *B. burgdorferi* sensu lato. The UPGMA tree was constructed with PAUP software and is based on a comparison of 344 bp of *ospC* genes. The tree was compared with the trees produced by neighbor-joining, maximum-likelihood, and parsimony methods with PAUP software. The four methods produced similar results. Scale bar represents calculated distance value. The abbreviations are defined in the legend to Fig. 2. A and E, America and Europe, respectively (these letters indicate the geographic origins of the isolates).

Greater genetic differences were observed among southern strains than northern strains in North America. Nineteen southern strains separated into all six clusters in the *B. burgdorferi* sensu stricto part of the tree and were dispersed into nine branches (Fig. 3). Southern *B. burgdorferi* sensu stricto strains occupied three branches. The first branch consisted of SI-1, SM-1, and SCI-2, which shared the cluster with North American and European *B. burgdorferi* sensu stricto strains. The second branch contained southern strains MI-2 and North American strain B31 as well as several European strains that have the same *ospC* sequence. The third branch was shared by southern *B. burgdorferi* sensu stricto strain MI-5, southern *B. bissettii* strains MI-9 and FD-1, and southern *B. andersonii* strains MOD-5 and MOK-3a. Southern *B. andersonii* strains BC-1, SI-10, and MOS-1b and southern *B. bissettii* strains SCW-30h and AI-1 occupy separate branches. However, southern *B. bissettii* strain MI-6 shared a branch with southern *B. andersonii* strain MOD-1. Interestingly, but not surprisingly, MI-8, as noted in our previous reports (35; Lin et al., Proc. VIII Int. Conf. Lyme Borreliosis Other Emerg. Tick-Borne Dis.), is present but distinct from *B. burgdorferi* sensu stricto strains. As noted earlier, its taxonomic status remains uncertain, but it appears to be an undescribed genospecies.

DISCUSSION

Usually, the greater the genetic diversity of a given species, the longer the time that was available for its evolution. When several phylogenetic trees derived from different genes or molecular markers are compared, if the topologies of the trees are

TABLE 4. Relationships between *ospC* major groups and their biological sources and geographic origins

Major *ospC* group	Strains	Biological sources[a]	Origins
A	HII, IP1, IP2, IP3, P1F, L5, TXGW	Patients (blood, CSF, synovial fluid, skin)	USA (New York, California, Texas, Florida)
	MI2	Animal (*P. gossypinus*)	Italy, France, Austria, Germany
	B31, CA4, Pka	Ticks (*I. scapularis, I. pacificus, I. ricinus*)	
B	61BV3, BUR, DK7, Pbre	Patients (skin)	USA (New York)
	35B808, ZS7, OC2	Ticks (*I. scapularis, I. ricinus*)	Germany, Denmark
C	OC3	Tick (*I. scapularis*)	USA (New York)
D	CA11.2A, OC4	Ticks (*I. scapularis, I. pacificus*)	USA (New York, California)
E	28691, N40, OC5, OC7	Tick (*I. scapularis*)	USA (New York, Pennsylvania)
F	27579, OC6, *B. pacificus*, Son188	Ticks (*I. scapularis, I. pacificus*)	USA (New York, Connecticut, California)
G	OC8	Tick (*I. scapularis*)	USA (New York)
H	OC9	Tick (*I. scapularis*)	USA (New York)
I	297, HB19, OC10	Patients (blood, CSF)	USA (New York, Connecticut)
		Tick (*I. scapularis*)	
J	MIL, OC11	Ticks (*I. scapularis, I. ricinus*)	USA (New York), Slovakia
K	272, 297, KIPP, MUL, 28354, OC12, OC13	Patients (CSF, skin)	USA (New York, Connecticut, Maryland)
		Tick (*I. scapularis*)	
L	21347, T255	Animal (*P. leucopus*)	USA (Wisconsin), Germany
		Tick (*I. ricinus*)	
M	2591	Animal (*P. leucopus*)	USA (Connecticut)
N	26815	Chipmunk	USA (Connecticut)
O	DUNKIRK	Patients (skin)	USA (New York)
P	20006	Tick (*I. ricinus*)	France
Q	212	Tick (*I. ricinus*)	France
R	ESP-1, NE56	Tick (*I. ricinus*)	Spain, Switzerland
S	Z136	Tick (*I. ricinus*)	Germany
T	OC16	Patient (EM)	USA (New York)
U	OC17	Patient (EM)	USA (New York)
V	S-1, SM-1, SC-12	Animal (*P. gossypinus*)	USA (Georgia)
W	DN127	Tick (*I. pacificus*)	USA (California)
X	25015	Tick (*I. scapularis*)	USA (New York)
Y	MI-5, MI-9, FD-1	Animal (*P. gossypinus, S. hispidus*)	USA (Florida)
Z	MI-8	Animal (*S. hispidus*)	USA (Florida)
A1	AI1	Animal (*S. hispidus*)	USA (Georgia)
B1	SCW-30h	Tick (*I. minor*)	USA (South Carolina)
C1	SCGT-8a, SCGT-10	Animal (*N. floridana*)	USA (South Carolina)
		Tick (*I. minor*)	
D1	MI6, MOD1	Animal (*S. hispidus*)	USA (Georgia)
		Tick (*I. dentatus*)	
E1	21123	Tick (*I. dentatus*)	USA (New York)
F1	BC1	Tick (*I. dentatus*)	USA (Georgia)
G1	MOS1b, SI-10	Ticks (*I. dentatus, I. scapularis*)	USA (Georgia, Missouri)
H1	MOD5	Tick (*I. dentatus*)	USA (Missouri)
I1	MOK3a	Tick (*I. dentatus*)	USA (Missouri)
J1	VS461	Tick (*I. ricinus*)	Switzerland
K1	20047	Tick (*I. ricinus*)	France
L1	VS116	Tick (*I. ricinus*)	Switzerland

[a] CSF, cerebrospinal fluid; EM, erythema migrans.

different, this may suggest that genetic transfer or recombination has occurred. The phylogenetic trees generated from the *ospC* gene of southern strains show greater differences than phylogenetic trees derived from the *rrf-rrl* intergenetic spacers (35), pulsed-field gel electrophoresis (39; Lin et al., Proc. VIII Int. Conf. Lyme Borreliosis Other Emerg. Tick-Borne Dis.), and randomly amplified polymorphic DNA fingerprint (Lin et al., Proc. VIII Int. Conf. Lyme Borreliosis Other Emerg. Tick-Borne Dis.).

Similarity matrixes, amino acid sequences, phylogenetic trees, and major *ospC* groups all suggest that lateral transfer and internal gene recombination of the *ospC* gene occurred between *B. burgdorferi* sensu stricto and *B. bissettii* or between *B. bissettii* and *B. andersonii*. OspC diversity may be promoted by several mechanisms, including host-stimulated immunological selection, gene transfer, intragenomic gene recombination, effects of environmental constraints, or other factors. Of these factors, frequency-dependent selection might be the major force maintaining the variation in *ospC* (21, 64).

The great diversity of OspC compared to OspA (a single type in *B. burgdorferi* sensu stricto and *B. afzelii*) (30, 70) is strong evidence that immunological selection and gene transfer happened frequently in *B. burgdorferi* sensu stricto and *B. afzelii*. This frequency-dependent selection is created by the host immune system, because OspC is a major protective antigen and is expressed on the surface of spirochetes during tick feeding (54). It can cause a strong immunoglobulin M response at an early stage of disease (19). Interestingly, OspC can protect animals against subsequent infection by the same strain (26) but not by heterologous strains (49). Also, injection with polyclonal and monoclonal antibodies to OspC can cure chronically infected mice and prevent reinfection with the same strain. It can also cure current infection (72). To escape selective immunological pressure caused by hosts, spirochetes can

change their *ospC* sequences and thus cause variation of the *ospC* gene.

In addition to immunological selection, there is another strategy for bacteria to escape immunological attack. The spirochetes which produce stable OspA are kept in the tick, and spirochetes which produce OspC or nothing are able to enter a host. Recent studies show that before the blood meal, spirochetes in the tick are a homogeneous population that mainly produce OspA. During the blood meal, the population becomes more heterogeneous. Many spirochetes produce both OspA and OspC, whereas others produce only a single Osp, and a few produce neither Osp. Most of the spirochetes in the salivary glands and host dermis produce neither OspA nor OspC, and a significant minority produce only OspC. OspC may play a role in allowing the spirochetes to escape from the gut into the hemocoele. OspA serves to bind spirochetes in the tick midgut, and OspC facilitates transfer from the gut lumen to the hemocoele (41). By downregulating OspA, the spirochetes might free themselves to migrate through the midgut epithelium and out of the midgut (26, 46, 59, 60). Spirochetes change their expression pattern of Osps in ticks when they move from the gut to the salivary gland and host dermis (41).

Isolate MI-8 was considered an undescribed genospecies in our previous report (35), and the current *ospC* analysis supports this conclusion. It occupies a separate branch in both of the phylogenetic trees (Fig. 2 and 3) derived from amino acid sequences and partial nucleotide sequences of *ospC*. It shares a branch with southern *B. bissettii* strains SCGT-8a and SCGT-10 in the tree derived from amino acid sequences (Fig. 2). In the tree generated from partial nucleotide sequences, it is related but distinct from *B. burgdorferi* sensu stricto strains 272, 28354, MUL, KIPP, 28691, and N40 and southern *B. bissettii* strains AI-1 and SCW-30h (Fig. 3). Whole DNA-DNA hybridization analysis with a group of strains containing MI-8 did not allow a clear conclusion because the hybridization levels were in borderline positions (Guy Baranton, Pasteur Institute, Paris, France, personal communication).

Since the North American *B. burgdorferi* sensu stricto strains in the clusters noted above were isolated from human skin, the major tick vector *I. scapularis*, or the common animal reservoir, *P. gossypinus*, perhaps the MI-8 strain and the southern *B. bissettii* strains SCW-30h and AI-1 in this cluster might be infective to humans (Fig. 3). Also needing further evaluation is another undescribed southern spirochete isolate, TXW-1. As noted in the Results, we were unable to amplify the *ospC* gene under various PCR conditions. Whether it lacks the *ospC* gene or the undetected gene in this isolate is truncated or otherwise altered at the primer sites remains to be determined. Although this strain appeared to be close to *B. garinii* in our previous analysis (35) using PCR-restriction fragment length polymorphism of the *rrf-rrl* intergenic spacer, data in the present study suggest that it belongs to an undescribed species among relapsing fever borreliae.

OspC might play a direct role in determining the potential for a given isolate to cause disseminated disease. The major *ospC* groups of *B. burgdorferi* sensu stricto found in human erythema migrans lesions are only a subset of the groups found in ticks based on single-strand conformation polymorphism analysis (27, 64). Moreover, of the 58 defined *ospC* groups in *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*, only 10 groups belong to known invasive *Borrelia* strains that are able to cause disseminated infection in humans (9, 21).

Infectivity and pathogenicity of most southern strains remain to be determined. Thus far, only *B. burgdorferi* sensu stricto has been confirmed to be pathogenic for humans in the United States. *B. andersonii* occurs in the eastern half of the United States and appears to exist primarily in an enzootic cycle involving cottontail rabbits and *I. dentatus*. Therefore, *B. andersonii* is thought not to infect humans. *B. bissettii* has been isolated only from ticks and nonhuman animals to date in the United States. Whether it is infectious or pathogenic for humans is unknown; however, several isolates identified as *B. bissettii* have been isolated from Slovenian patients (46). This suggests that *B. bissettii* might cause human Lyme disease in the United States. Earlier, strain 25015 from New York, the reference strain for this genospecies, was thought to be infectious but nonpathogenic in mice (3). However, a later study reported it to cause mild arthritis (23).

Typical and atypical *B. burgdorferi* sensu stricto strains are found among southern isolates (Fig. 2 and 3). Southern *B. burgdorferi* sensu stricto isolates appear in almost all of the branches in the North American part of the tree. This indicates great genetic diversity among southern *B. burgdorferi* sensu stricto. Typical southern *B. burgdorferi* sensu stricto strains, such as MI-2, are quite similar to the B31 reference strain and are located in the same branch (Fig. 2 and 3). Previous analyses based on *rrf-rrl* intergenic spacer sequences indicate the same association (35). Except for B31 which was isolated from the tick *I. scapularis*, all the strains in this branch of the tree were isolated from cerebrospinal fluid, blood, synovial fluid, and skin of patients in Europe (Fig. 2 and 3). MI-2 most closely aligns with a group that possesses many proven human-invasive strains (9, 57). TXGW and MI-2 are currently the only two known southern isolates that belong to this group.

The *ospC* genotype is associated with invasiveness and infectivity of *B. burgdorferi* and might determine the difference in pathogenicity between clones (9, 57). A given strain is defined as invasive if it is isolated from a secondary site in the body, for example, blood, joints, or the central nervous system. Although OspC is not expressed as a major protein in *B. burgdorferi* in culture, it is an immunodominant protein of the early humoral immune response in humans (1, 19, 65, 67; B. Wilske, V. Preac-Mursic, G. Schierz, G. Liegl, and W. Gueye, Proc. Lyme Borreliosis Update Europe, p. 299–309, 1987) and is a specific and sensitive marker for the early stage of Lyme disease (43, 68, 69). The expression pattern of *ospC* plays a role in the infection cycle of the spirochete (54, 59–61). *ospC* is expressed and *ospA* is repressed when the ticks begin feeding on mice and humans (24, 54). Factors that regulate the switch from expression of OspA to OspC are likely varied and complex. The switch is in part due to the change in temperature; *ospC* is induced at 32 to 37°C, but not at 24°C (54, 59–61). Other factors, such as cell density (18), growth phase (50), or a change in pH (14, 15), involve the expression regulation of OspC and OspA. OspC expression was shown to increase in cultures treated with hemolymph (31). Evidence suggests coregulation of the *ospA* and *ospC* genes (33).

The major *ospC* groups found in human erythema migrans lesions are only a subset of the *ospC* groups found in ticks (57). Moreover, of the 22 major *ospC* groups in *B. burgdorferi* sensu

stricto, only four groups (A, B, I, and K) had higher frequencies in the primary infected sites than in ticks and were the only groups found in secondary sites. The frequency distribution of *ospC* groups from ticks is significantly different from that from primary sites (erythema migrans lesion or skin rash), which in turn is significantly different from that in secondary sites (blood, joints, or the central nervous system) (57). Similarly, of the 14 *ospC* major groups in *B. afzelii*, only 2 groups contain the putative invasive isolates, and of 22 *ospC* groups in *B. garinii*, 4 groups contain all the putative invasive isolates (9, 57). Thus, there are only a minority (10 of 58) of *ospC* groups in the three genospecies (*B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*) that are invasive and able to colonize and persist in organs other than skin. Other groups may cause erythema migrans but do not appear to be invasive and disseminate to other organs.

Twelve major *ospC* groups are recognized among our 19 southern strains analyzed here. Likewise, of the 12 *ospC* groups, only one is considered to be in the invasive group (A). Southern *B. burgdorferi* sensu stricto strain MI-2, isolated from a cotton mouse from Florida, most closely aligns with a group that possesses many proven human invasive strains. Transmission experiments showed that this group of southern spirochetes is able to infect mice and hamsters and that *I. scapularis* can transmit it (J. H. Oliver et al., unpublished data). This suggests that typical *B. burgdorferi* sensu stricto strains with strong virulence and invasiveness exist in certain enzootic cycles in the southern United States and that MI-2 might be able to cause invasive human Lyme disease. Other southern *B. burgdorferi* sensu stricto strains, such as SI-1, SM-1, and SCI-2, are genetically closely related to North American *B. burgdorferi* sensu stricto strain 21347 (L) and European *B. burgdorferi* sensu stricto strain T255 (*ospC* group L), from the white-footed mouse and *I. ricinus*, respectively.

The diversity of *ospC* in one local population in New York was almost as great as the diversity worldwide (64). However, our results suggest that the distribution of *ospC* major groups might be associated with geographic sites. Of the three *ospC* groups containing *B. burgdorferi* sensu stricto isolates, two of them (SI-1 group and MI-5 mixture group) separated into different branches in the tree and formed different *ospC* groups (Fig. 2, Table 4).

B. andersonii and *B. bissettii* strains were distributed throughout the clusters in the *B. burgdorferi* sensu stricto part of the tree, suggesting that these three genospecies are closely related (Fig. 3). Perhaps genetic transfer or intragenomic gene recombination occurred among southern strains and also between southern and northern strains. Several methods can be used to determine genetic transfer or recombination based on sequence data (21). The first method is to compare several gene trees generated from the same set of strains but different molecular markers; a difference in branching order is evidence for recombination. Comparison of the phylogenetic trees generated from *rrf-rrl* intergenic spacer, *fla*, *rrs*, pulsed-field gel electrophoresis, and random amplified polymorphic DNA shows that the phylogenetic trees generated from the *ospC* data are different from the trees derived from others. This suggests that lateral transfer of the *ospC* gene or intragenomic gene recombination occurred between *B. burgdorferi* sensu stricto and *B. bissettii* and between *B. bissettii* and *B. andersonii*, as mentioned in the Results.

Another method is to find significant similarities in sequences among different genospecies. Close identity was observed between the *ospC* sequences of *B. burgdorferi* sensu stricto and *B. bissettii* and between *B. bissettii* and *B. andersonii*. All the differences between the phylogenetic trees generated from *ospC* data and the trees generated from other genes and the close identity of *ospC* sequences among genospecies are caused by the introduction of a small piece of DNA (about 500 bp) into the central variable region of the *ospC* gene (Table 3).

Southern *B. burgdorferi* sensu stricto strain MI-5 shared a branch with southern *B. bissettii* strains MI-9 and FD-1, and this branch combined with southern *B. andersonii* strains MOD-5 and MOK-3a (Fig. 3). They were located in the fourth cluster of the tree, which contained most of the typical North American and European *B. burgdorferi* sensu stricto strains. This distribution adds additional evidence suggesting that the three southern *B. burgdorferi* genospecies are closely related. The evolutionary process continues, and the different levels of infectivity and virulence among the strains may be due to selection pressures in the tick vectors or animal hosts or to environmental conditions.

It is not strange that the southern *B. andersonii* strains MOD-1, BC-1, SI-10, and MOS-1b shared the same cluster as *B. andersonii* strains 21123 and 19857 from the north. All of them belong to the *B. andersonii* genospecies and were isolated from *I. dentatus* and/or cottontail rabbits except for SI-10, which was isolated from *I. scapularis*. Unexpectedly, however, they were grouped into the same cluster as the southern *B. bissettii* strain MI-6 and the North American *B. burgdorferi* sensu stricto strains 27579, *B. pacificus* (strain name, not genospecies name), SON188, and the European *B. burgdorferi* sensu stricto strain MIL (Fig. 3). The strains in the major *ospC* groups F, H, J, T, and U also grouped into this cluster (Fig. 3). Except for MI-6, which was isolated from a cotton rat in Florida, all these strains were isolated from ticks in the *I. ricinus* species complex. *B. pacificus* and SON188 were isolated from *I. pacificus* ticks in California, 27579 was isolated from *I. scapularis* in Connecticut, MIL was isolated from *I. ricinus* in Slovakia, and strains in *ospC* groups F, H, J, T, and U were isolated from *I. scapularis* from New York. The data suggest that the tick vector plays a role in the evolutionary process of the *ospC* gene. This interaction is probably influenced by geographic location and the genospecies distribution of *Borrelia* strains. That is probably why *Borrelia* strains from different genospecies and different geographic locations occupy distinct branches in the same cluster in phylogenetic trees (Fig. 2 and 3).

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