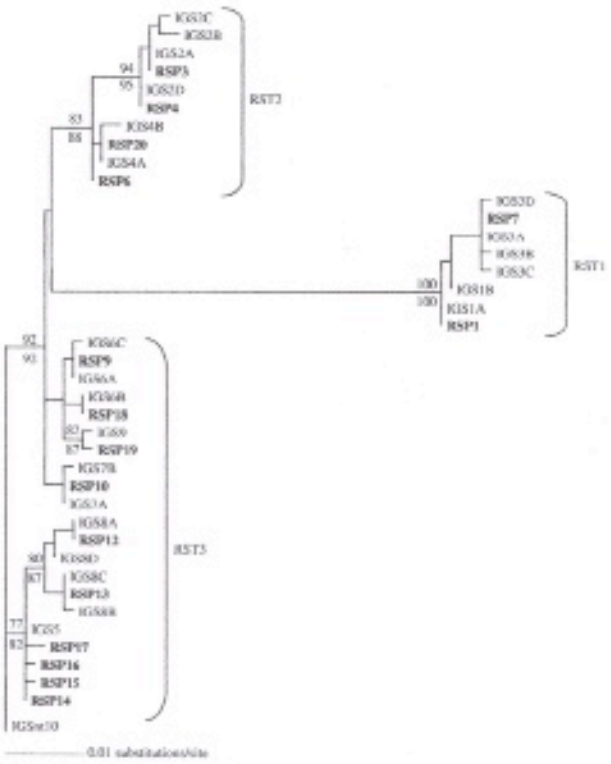

FIG. 1. Unrooted maximum-likelihood phylogenetic tree based on *rrs-rrlA* data set and GenBank reference strains. Nonparametric bootstrap values for nodes with >70% support in both maximum-likelihood and maximum-parsimony analyses are above and below the branches, respectively. *B. burgdorferi* RSP alleles identified in this study are in bold.

but parsimony uninformative, and 205 were parsimony informative. The mean nucleotide diversity per position (π) was 0.193 (Table 1). The lengths of the nucleotide sequences varied from 498 to 510, coding for 166 to 170 amino acids. Of the variable sites, 162 were at first and/or second codon positions, and 110 were at third codon positions. Pairwise nucleotide sequence identity among *ospC* alleles ranged from 74.0 to 87.6%, corresponding to amino acid identities of 63.9 and 80.2%, respectively. To assess the placement of *ospC* alleles found in this study with previously published *ospC* major groups, we compared sequence differences among our *ospC* alleles and major *ospC* groups found worldwide. It has been suggested that the members of the same *ospC* group will have a sequence difference of <2% and members of different groups >8% (46). By this criterion, 15 of the *ospC* allele types observed in this study fell into 15 major *ospC* groups previously found in the northeastern United States (Table 2). This relationship was further supported by maximum-likelihood and maximum-parsimony phylogenetic analyses that provided high bootstrap values for terminal nodes with *ospC* alleles and previously identified *ospC* groups (Fig. 2). The exception was *ospC* allele type 16 (AT16), which did not cluster with any *ospC* group and whose sequence was 85% identical to the closest *ospC* allele (*ospC* AT10) identified in this study. Furthermore, this allele was more than 8% different from any *ospC* allele submitted to GenBank to date.

Relationship between *rrs-rrlA* and *ospC* alleles and comparison with other typing methods. The partial sequences of the chromosomal noncoding *rrs-rrlA* and nearly complete sequences of *ospC*, located on circular plasmid cp26, were, without exception, strongly associated in the present data set. Sixteen unique biallelic profiles were resolved, with each profile represented by a unique combination of *rrs-rrlA* and *ospC* alleles. RFLP analyses of samples used in the present study showed that the RST1 genotype was linked with two *ospC*/*rrs-rrlA* allele profiles, RST2 with 4 and RST3 with 10 *ospC*/*rrs-rrlA* allele profiles (Table 2). RST1 and RST2 each formed well-supported clusters in the *rrs-rrlA* tree. In contrast, RST3 types were more diverse (Fig. 1). Each of the *rrs-rrlA* alleles corresponded to at least one of nine previously identified IGS types (3). The resolution of linkage among *rrs-rrlA* and *ospC* groups observed in the present study, however, was not apparent in the IGS typing framework. For example, *ospC* groups K and H were exclusively associated with RSP alleles 3 and 4, respectively, in the present study, but both belonged to the same IGS type (IGS2). Similarly, *ospC* groups U and T were exclusively associated with RSP alleles 12 and 13, respectively, but both belong to IGS type 8 (3) (Table 2).

Comparison of tree topologies. To determine whether the linkage among genetic loci could be explained by a clonal model, phylogenetic trees were constructed for each locus and examined for congruence. The Shimodaira-Hasegawa test was used to determine whether the same or different phylogenetic information was obtained from the analysis of *rrs-rrlA* and *ospC*. The *rrs-rrlA* phylogenetic tree was incongruent with the *ospC* tree (Shimodaira-Hasegawa test, $P < 0.05$) (Fig. 3). Some

TABLE 2. Comparison of *rrs-rrlA* and *ospC* alleles with different typing systems

rrs-rrlA IGS				*ospC*	
RSP[a] (no. of isolates sharing the same allele)	IGS type[a]	IGS subtype[a]	RST[b]	*ospC* AT[c]	*ospC* group[c]
1 (12)	1	1A	1	1	A
3 (25)	2	2A	2	11	K
4 (7)	2	2D	2	8	H
7 (11)	3	3A	1	2	B
6 (7)	4	NI[d]	2	6	F
20 (15)	4	4A	2	13	N
14 (4)	5	NI	3	4	D
15 (3)	5	NI	3	3	C
16 (3)	5	NI	3	10	J
17 (1)	5	NI	3	16	NT[e]
9 (7)	6	6A	3	12	M
18 (6)	6	6B	3	7	G
10 (12)	7	7A	3	9	I
12 (6)	8	8A	3	15	U
13 (2)	8	8C	3	14	T
19 (6)	9	NI	3	5	E

[a] *rrs-rrlA* typing based on reference 3.
[b] *rrs-rrlA* typing based on reference 20.
[c] *ospC* groups according to references 34 and 46.
[d] NI, not identified.
[e] NT, new type.
[f] *rrs-rrlA* typing based on this study.
[g] *ospC* typing based on this study.

Table S1. Accession numbers of existing genome, cp26, and *ospC* sequences of *B. burgdorferi* and *B. bissettii* by strain, location, and genotype

Strain	Location	Accession numbers		*ospC* genotype	*ospC* seq.
		Genome seq.	cp26 seq.		
B31	Northeast	AE000783	AE000792	A	X69596
ZS7	Europe	CP001205	CP001212	Ba	EF537413
64b	Northeast	ABKA00000000	CP001422	Bb	NC_011724
WI91-23	Midwest	ABJW00000000	CP001446	C	DQ437462
29805	Northeast	ABJX00000000	CP001550	Da	AF029863
94a	Northeast	ABGK00000000	CP001493	Db	GQ478283
72a	Northeast	ABGJ00000000	CP001375	E	AY275221
118a	Northeast	ABGI00000000	CP001535	Fa	AY275225
CA-11.2a	California	ABJY00000000	CP001484	Fb	EF537433
156a	Northeast	ABCV00000000	CP001271	Fc	GQ478285
				G	AY275223
				Ha	EU377781
				Hb	GQ478286
				Ia	AY275219
				Ib	EU377752
				J	CP001535
				K	AY275214
				L	EU375832
				M	CP001550
				N	EU377775
				O	FJ997281
				T	AY275222
				Ua	EU377769
				Ub	GQ478287
				A3	EF592541
				B3	EF592542
				C3	EF592543
				D3	EF592544
				E3	EF592545
				F3	EF592547
				H3	FJ932733
				I3	FJ932734
				B. bissettii 25015	U04282