Phylogenetic Analysis of *Borrelia burgdorferi* Sensu Stricto by Arbitrarily Primed PCR and Pulsed-Field Gel Electrophoresis

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Borrelia burgdorferi sensu lato is present all over the world. However, most species belonging to this complex have a limited distribution; the only exception is *B. burgdorferi* sensu stricto, which is encountered both in Europe and in the United States. The aim of this study was to clarify the way that *B. burgdorferi* sensu stricto migrated. To do this, we compared the genetic polymorphism of 33 *B. burgdorferi* sensu stricto strains isolated in Europe and 28 strains isolated in the United States by using both pulsed-field gel electrophoresis and arbitrarily primed PCR. Phylogenetic analysis of each of the two sets of data was performed by a genetic distance method and a parsimony method. The results were consistent and revealed that the American strains were more heterogeneous than the European strains. The hypothesis that *B. burgdorferi* sensu stricto was introduced to Europe from America rather than vice versa has to be considered.

Borrelia burgdorferi sensu lato is a spirochete that is responsible for Lyme borreliosis, a disease with a wide spectrum of symptoms, ranging from minor cutaneous erythema to severe arthritis or neurological manifestations. This worldwide disease is transmitted to humans by Ixodidae ticks. The heterogeneous clinical presentation of the disease has been linked to the genetic diversity of the *B. burgdorferi* sensu lato complex, which has been divided into at least eight species or genomic groups (22). *Borrelia garinii* and *Borrelia afzelii* are encountered in Europe and Asia, whereas *B. burgdorferi* sensu stricto is present in the United States and in Europe but is absent from Asia (19, 33). Until recently, *B. burgdorferi* sensu stricto was considered the only species that occurs in North America. Several genetic studies have shown that *B. burgdorferi* sensu stricto strains isolated from both the Old World and the New World exhibit a high degree of similarity. There is a question concerning the direction of migration of *B. burgdorferi* sensu stricto strains. Two hypotheses can be proposed. One hypothesis is that a long time ago, an ancestor strain of *B. burgdorferi* sensu stricto migrated to America from Europe, where it had evolved for a long time. The ability of migratory birds to spread *Borrelia* strains (21) makes such a migration likely. This hypothesis is supported by the existence of several highly divergent species in Eurasia compared with an apparent unicity in America, suggesting that the whole Lyme borreliosis complex originated in Eurasia. However, the discovery of at least two other genospecies in the United States, *Borrelia andersonii* (20) and group DN127, led us to examine whether *B. burgdorferi* sensu stricto could have originated in America before it migrated to Europe.

In order to further investigate the evolution of *B. burgdorferi* sensu stricto and the migration route from west to east or vice versa, two methods, pulsed-field gel electrophoresis (PFGE) and arbitrarily primed PCR (AP-PCR) were used to compare the genetic polymorphism of European and American strains.

MATERIALS AND METHODS

Bacterial strains. The 61 *B. burgdorferi* sensu stricto isolates used in this study are listed in Table 1; 33 of these strains were isolated in Europe, and 28 strains were isolated in North America. The organisms were chosen from our collection so that the greatest possible geographic diversity for each continent was represented. Strain DN127, a member of genomic group DN127, was used as an outgroup strain for phylogenetic studies. The isolates were grown in BSK-H medium (Sigma) supplemented with 6% rabbit serum at 30°C (4).

PFGE. Previously described procedures were used to prepare high-molecular-weight genomic DNAs and for PFGE (6, 7, 9). Separation of DNA fragments was achieved with a pulse time ramped from 3 to 50 s for 30 h with a contour-clamped homogeneous electric field model DR11 apparatus (Bio-Rad Laboratories, Richmond, Calif.). To separate restriction fragments shorter than 50 kbp, we used zero-integrated-field electrophoresis (ZIFE) with the AutoBase system (Techgen Company, Les Ulis, France). The ROM card allowing separation of fragments ranging in size from 8 to 50 kbp was used. Rare cutting endonucleases *MluI*, *BssHII*, and *SacII* were used to differentiate isolates. Lambda concatamers (monomer size, 48.5 kbp) purchased from New England Biolabs, Beverly, Mass., and contour-clamped homogeneous electric field DNA size standards (8 to 48 kbp; Bio-Rad) were used as size markers with the Bio-Rad apparatus and the ZIFE apparatus, respectively. Strains which exhibited the same PFGE pattern with *MluI*, the same PFGE pattern with *BssHII*, and the same PFGE pattern with *SacII* were placed in the same PFGE polymorphism group; different groups were designated groups A to S.

DNA blot analysis. For blot experiments as described by Southern (25), DNA fragments separated by PFGE were subjected to depurination and transferred to Hybond-N nylon membranes (Amersham). To prepare a probe, the chromosomal band from isolate 212 was excised from a low-melting-temperature agarose pulsed-field gel, washed twice in TE (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), and digested with *MluI* or *BssHII* as described above. Labelling was performed by random priming with digoxigenin-11-dUTP as previously described (5). Hybridized fragments were revealed with an anti-digoxigenin antibody conjugated with alkaline phosphatase and CSPD, a chemoluminescent substrate, by using the procedure recommended by the manufacturer (Boehringer). The membranes were exposed for 15 min.

DNA preparation. Isolates in BSK-H medium were incubated at 30°C for 7 to 12 days until the density was about 10^8 cells per ml. Cells were harvested, washed in phosphate-buffered saline, resuspended in distilled water, and finally boiled at 110°C for 15 min. Thermolysates were stored at -20°C before they were used for AP-PCR experiments.

AP-PCR fingerprinting. Fingerprinting was performed as described previously (31), with minor modifications. The reaction mixtures (25 µl) contained 5 µl of bacterial thermolysate, 1× *Taq* polymerase buffer (10 mM Tris [pH 8.3], 50 mM KCl, 4 mM $MgCl_2$), each deoxynucleoside triphosphate at a concentration of 0.2 mM, single oligonucleotide primers at concentrations of 1 to 5 µM, and 0.625 U of *Taq* polymerase (Amersham), and 4 µCi of [α - ^{32}P]dATP (ICN Pharmaceuticals, Inc., Costa Mesa, Calif.) was added to each mixture. The following primers were purchased from Genset, Paris, France: RSP (5'-GGAAACAGCTATGAC CATGA-3') (6), SP (5'-TTGTAAAACGACGGCCAG-3') (6), TSB (5'-AATG CTCTACCAACTGA-3') (7), T3 (5'-GCAATTAACCTCACTAAAG-3') (7), and OspC1 (5'-AGTGCATATT(AG)ATGACTTT-3'). The reaction mixtures were subjected twice to the following low-stringency temperature profile

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TABLE 1. Strains used in this study

Isolate	PFGE polymorphism group	Lane(s) on AP-PCR gel ^a	Geographical origin	Source	Person who provided
Charlie tick	A	63	Wisconsin	<i>Ixodes scapularis</i>	C. Kodner
Veery	B	42	Connecticut	Veery bird	R. T. Marconi
297	C	48	Connecticut	Human cerebrospinal fluid	
NY13-87	C	26	New York	Human skin	R. T. Marconi
VS 2	C	43	Shelter Island, N.Y.	<i>Ixodes scapularis</i>	O. Péter
Sh2-82	C	34	Texas	<i>Ixodes scapularis</i>	S. Bergström
CA2-87	D	21	California	<i>Ixodes pacificus</i>	R. T. Marconi
HUM 115	D	27	California	<i>Ixodes pacificus</i>	M. Janda
HUM 7814	E	8	California	<i>Ixodes pacificus</i>	M. Janda
HB19	E	37	Connecticut	Human blood	A. Barbour
CA6	F	23	California	<i>Ixodes pacificus</i>	R. Lane
MEN115	F	59	California	<i>Ixodes pacificus</i>	M. Janda
HUM 3336	F	60	California	<i>Ixodes pacificus</i>	M. Janda
CA5	F	61	California	<i>Ixodes pacificus</i>	R. Lane
SON 188	G	15	California	<i>Ixodes pacificus</i>	M. Janda
LAKE 339	G	52	California	<i>Ixodes pacificus</i>	M. Janda
NY1-86	H	51	New York	Human skin	R. T. Marconi
SON 2110	I	16	California	<i>Ixodes pacificus</i>	M. Janda
21343	J	9	Wisconsin	<i>Peromyscus leucopus</i>	J. F. Anderson
21305	K	50	Connecticut	<i>Peromyscus leucopus</i>	J. F. Anderson
SON 335	L	10	California	<i>Ixodes pacificus</i>	M. Janda
SON 328	L	19	California	<i>Ixodes pacificus</i>	M. Janda
SV1	M	49	France	<i>Ixodes ricinus</i>	C. Perez
35B808	M	33	Germany	<i>Ixodes ricinus</i>	A. Schönberg
HII	N	47	Italy	Human blood	V. Sambri
DK7	O	5	Denmark	Human skin	M. Theisen
SV4	O	25	France	<i>Ixodes ricinus</i>	C. Perez
212	O	39	France	<i>Ixodes ricinus</i>	F. Milward
20006	O	44	France	<i>Ixodes ricinus</i>	J. F. Anderson
GeHo	O	20	Germany	Human skin	Vögel
61BV3	O	28	Germany	Human skin	A. Schönberg
ZS7	O	31	Germany	<i>Ixodes ricinus</i>	L. Gern
Z118	O	45	Germany	<i>Ixodes ricinus</i>	A. Vogt
ESP-1	O	7	Spain	<i>Ixodes ricinus</i>	R. C. Johnson
IRS	O	24	Switzerland	<i>Ixodes ricinus</i>	
NE493	O	29	Switzerland	<i>Ixodes ricinus</i>	L. Gern
NE456	O	30	Switzerland	<i>Ixodes ricinus</i>	L. Gern
MIL	P	18	Slovakia	<i>Ixodes ricinus</i>	A. Livesley
Y1	Q	14	Finland	<i>Ixodes ricinus</i>	J. Tuomi
SV3	Q	62	France	<i>Ixodes ricinus</i>	C. Perez
Z136	Q	11	Germany	<i>Ixodes ricinus</i>	A. Vogt
NE196	Q	13	Switzerland	<i>Ixodes ricinus</i>	L. Gern
NE56	R	12	Switzerland	<i>Ixodes ricinus</i>	L. Gern
Holzer	S	1, 2	Austria	Human blood	G. Stanek
Lenz	S	3	Austria	Human heart muscle	G. Stanek
H3	S	22	Austria	Human skin	G. Stanek
T	S	35	Austria	Human skin	G. Stanek
H1	S	41	Austria	Human skin	G. Stanek
L5	S	46	Austria	Human skin	G. Stanek
P1F	S	4	Austria	Human synovial fluid	G. Stanek
Hb1	S	32	Austria	Mouse	G. Stanek
IP2	S	55	France	Human cerebrospinal fluid	G. Baranton
IP3	S	36	France	Human cerebrospinal fluid	G. Baranton
IP1	S	54	France	Human cerebrospinal fluid	G. Baranton
PKa	S	40	Germany	<i>Ixodes ricinus</i>	V. Preac Mursic
B31 ^T	S	6	New York	<i>Ixodes scapularis</i>	
19535	S	58	New York	<i>Peromyscus leucopus</i>	J. F. Anderson
26816	S	56	Rhode Island	<i>Microtus pennsylvanicus</i>	J. F. Anderson
27985	S	57	Shelter Island, N.Y.	<i>Ixodes scapularis</i>	J. F. Anderson
Cat Flea	S	53	Texas	<i>Ctenocephalides felis</i>	D. Ralph
1352	S	17	Texas	<i>Ixodes scapularis</i>	R. T. Marconi
DN127		38	California	<i>Ixodes pacificus</i>	M. Janda

^a See Fig. 3.

FIG. 1. (A) *Mlu*I restriction profiles of *B. burgdorferi* sensu stricto strains obtained with the Bio-Rad apparatus with the pulse time ramped from 3 to 50 s for 30 h. (B) Blot of the PFGE gel hybridized with the chromosomal fragment from *B. burgdorferi* 212 DNA. The lanes contained lambda concatemers (lanes 1, 16, and 30) and DNAs from isolates Cat Flea (lane 2), L5 (lane 3), T (lane 4), Hb1 (lane 5), B31^T (lane 6), CA5 (lane 7), MEN115 (lane 8), HUM 3336 (lane 9), 212 (lane 10), HB19 (lane 11), HUM 7814 (lane 12), NY13-87 (lane 13), Sh2-82 (lane 14), NE493 (lane 15), 61BV3 (lane 17), MIL (lane 18), 20006 (lane 19), Z118 (lane 20), SON 328 (lane 21), SON 335 (lane 22), SV3 (lane 23), Z136 (lane 24), 21305 (lane 25), LAKE 339 (lane 26), NY1-86 (lane 27), SON 188 (lane 28), and Veery (lane 29).

cycle: denaturation at 94°C for 5 min, low-stringency annealing at 40°C for 5 min, and extension at 72°C for 5 min. These cycles were followed by 40 high-stringency temperature profile cycles (94°C for 1 min, 60°C for 1 min, and 72°C for 2 min). Thermocycling was performed with a model OmniGene apparatus (Hybaid).

Each reaction mixture was combined 1:1 (vol/vol) with 98% formamide dye and heated to 95°C for 15 min before it was loaded onto a 6% acrylamide–50% urea sequencing gel. Electrophoresis was carried out at 1,800 V and 80 W for 3.5 h. The gels were dried and exposed for 24 h.

FIG. 2. *Mlu*I restriction profiles of the strains in Fig. 1 obtained by the ZIFE method with the 100- to 150-kbp ROM card. The lanes contained lambda concatemers (lanes 1, 17, and 30) and DNAs from isolates Cat Flea (lane 2), L5 (lane 3), T (lane 4), Hb1 (lane 5), B31^T (lane 6), CA5 (lane 7), MEN115 (lane 8), HUM 3336 (lane 9), 212 (lane 10), HB19 (lane 11), HUM 7814 (lane 12), NY13-87 (lane 13), Sh2-82 (lane 14), NE493 (lane 15), 61BV3 (lane 16), MIL (lane 18), 20006 (lane 19), Z118 (lane 20), SON 328 (lane 21), SON 335 (lane 22), SV3 (lane 23), Z136 (lane 24), 21305 (lane 25), LAKE 339 (lane 26), NY1-86 (lane 27), SON 188 (lane 28), and Veery (lane 29).

Phylogenetic analysis. To analyze the fingerprint data, we used the following three phylogenetic inference packages: PAUP (phylogenetic analysis using parsimony) (26), PHYLIP (phylogeny inference package) (10), and MEGA (molecular evolutionary genetics analysis) (17). The fingerprint data were collected and analyzed in blind experiments. Strain DN127, a member of genomic group DN127, was found to be closely related to *B. burgdorferi* sensu stricto (level of DNA relatedness, 57 to 67%; difference between the melting temperature of the homologous hybrid and the melting temperature of the heterologous hybrid, 5 to 7°C) (22). Thus, strain DN127 was used as an outgroup in the phylogenetic analysis.

RESULTS AND DISCUSSION

Genomic fingerprinting by PFGE. Because the *B. burgdorferi* genome has a G+C content of 27 to 30 mol% (3, 14), restriction endonucleases with G · C-rich recognition sequences were used to cut the DNA into sufficient numbers of fragments to allow interpretable comparisons. Restriction endonucleases *Mlu*I, *Bss*HII, and *Sac*II were chosen to do this since they had generated great polymorphism among *B. burgdorferi* sensu stricto strains previously (7, 8). The *Mlu*I patterns obtained under two different conditions of electrophoresis are shown in Fig. 1 and 2. When the model DRII apparatus (Bio-Rad) was used, the large fragments (fragments larger than 300 kbp) were well resolved (Fig. 1A). A 100- to 150-kbp ROM card was used to separate the 100- to 200-kbp fragments (Fig. 2). Fragments smaller than 50 kbp were visualized by using the 8- to 50-kbp ROM card (data not shown). However, in the low-molecular-weight range, the presence of plasmids made interpretation of the PFGE patterns difficult. Thus, it was impossible to distinguish whether DNA fragments smaller than 80 kbp were intact or plasmid fragments or cut chromosomes. To answer this question, each PFGE gel was transferred to a nylon membrane and hybridized with a chromosomal probe prepared from strain 212 (Fig. 1B). When this was done, the chromosomal fragments were easily recognized. However, this method did not allow us to visualize chromosomal fragments smaller than 10 kbp. Using *Mlu*I, *Bss*HII, and *Sac*II, we obtained 9, 12, and 6 restriction patterns, respectively. The sizes of the restriction fragments obtained after digestion by the three enzymes are shown in Table 2. An analysis of all of the results allowed us to define 19 polymorphism groups, which were designated groups A to S (Table 1), for the 61 *B. burgdorferi* sensu stricto strains studied. These results confirmed the high degree of heterogeneity of genetic patterns for *B. burgdorferi* sensu stricto reported previously (7). A total of 12 of the 19 groups contained only American strains (groups A to L), 6 groups contained only European strains (groups M to R), and 1 group contained both American and European strains (group S). Moreover, 24 of the 33 European strains studied belonged to only two groups. Clearly, these results confirmed the assumption that American strains are more heterogeneous than European strains.

The size of the chromosome deduced from the analysis of *Mlu*I and *Sac*II fragments was 935 to 950 kbp, which was completely consistent with previously published results (6, 11). After restriction by the three enzymes, a genetic map was constructed (data not shown), and it was noteworthy that a supplementary 10- to 15-kbp fragment was found on the left-end fragment on the chromosome maps of strains belonging to American polymorphism group F. Moreover, variation in the length of the right-end fragment of 18, 24, or 33 kbp according to the polymorphism group was also observed when the *Bss*HII fragments were analyzed. However, all of the European polymorphism groups were homogeneous in this respect (all had a 24-kbp fragment). Considering the linear structure of the chromosome in the genus *Borrelia*, this variation in length at the extremities could be linked to the mechanism of replication of the spirochetes.

TABLE 2. Sizes of fragments obtained after restriction digestion by three enzymes of DNAs of the *B. burgdorferi* sensu stricto isolates used in this study

[illegible]

FIG. 3. AP-PCR fingerprints of the *B. burgdorferi* sensu stricto strains listed in Table 1 obtained with primer T5B. The arrows indicate polymorphic characters used to construct the data matrix.

Genomic fingerprinting by AP-PCR. AP-PCR was developed by Welsh et al. (30) to generate polymorphic fingerprints in order to distinguish variability between strains within a species or between strains belonging to closely related species. Discrete fingerprints reflect sequence differences scattered on the genome and therefore can be used to measure genetic distances and to establish phylogenetic relationships at an interspecific level or, especially, at an intraspecific level. This approach was used previously to resolve three distinct phyletic groups in *B. burgdorferi* sensu lato, corresponding to the three main species, *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* (31). In this study AP-PCR was used to determine the intraspecific variation in the patterns, which allowed us to compare the polymorphism of *B. burgdorferi* sensu stricto strains from Europe and from the United States.

The AP-PCR fingerprints of DNAs from *B. burgdorferi* sensu stricto strains consisted of reproducible sets of products. To increase the validity of the results, the same DNA preparations were studied concurrently with each primer, and all AP-PCR products obtained with one primer were run on the same gel. It has been shown previously that AP-PCR fingerprints markedly differ from one species to another, which led to the recognition of *Borrelia* species (31). In contrast, within a species, *Borrelia* strains shared a high number of fingerprints. However, about 10 polymorphic characters per primer were available to compare strains. The fingerprints obtained from 62 strains with primer T5B are shown in Fig. 3. Since no extensive genetic exchange has been demonstrated so far in *B. burgdorferi* sensu lato except for the plasmidic *ospC* gene (15, 19), phylogenetic

conclusions may be deduced from the shared derived characters observed for *B. burgdorferi* sensu stricto.

Phylogenetic analysis. All PFGE and AP-PCR data were analyzed by both genetic distance and parsimony methods.

(i) PFGE data. The data obtained for all restriction fragments on the gels were transformed into a binary data matrix, in which each fragment was scored as 1 (present) or 0 (absent). Thus, the data from PFGE experiments in which enzymes *Mlu*I, *Bss*HII, and *Sac*II were used yielded a matrix of 62 strains $\times$ 49 characters. In order to reduce the size of the data matrix and to facilitate the phylogenetic distance analysis, only one strain of each group was selected and a minimatrix was used to construct trees by genetic distance methods.

We used the Fitch+ genetic distance algorithm (13) in the PHYLIP phylogenetic analysis package to construct trees from PFGE pairwise distance measurements. This algorithm estimates phylogeny from distance matrix data by minimizing the differences between observed and expected pairwise distances (12). The tree which was obtained is shown in Fig. 4. The sum of squares for this tree is 9.8, and the average standard deviation is 16%. An extremely similar tree was obtained by using the neighbor-joining method (23) in the MEGA software (data not shown).

Figure 5 shows a phylogenetic tree estimated by parsimony analysis with the PAUP program. Parsimony methods search for minimal-length trees (i.e., trees that minimize the number of evolutionary changes needed to explain the available data under a prespecified set of constraints upon permissible character changes). This program was used in the heuristic search

FIG. 4. Phylogenetic tree based on genetic distances and constructed by using the Fitch+ genetic distance method in the PHYLIP package. The tree was generated from a reduced distance matrix for 20 taxa, each of which represented a PFGE polymorphism group. The European strains are underlined. The number of isolates in each group is indicated in brackets.

mode. The tree obtained was similar to the tree obtained by genetic distance analysis, except that polymorphism groups C, D, F, I, L, N, and S clustered in branch II instead of branch I as in the distance analysis. The consensus tree obtained by using the Mix+ parsimony algorithm in PHYLIP (data not shown) was similar to the tree obtained by using the parsimony analysis procedure in PAUP.

(ii) **AP-PCR data.** Each fragment on the gels was scored as 1 (present) or 0 (absent). Only unambiguous fragments were scored. Using five primers and 62 isolates, we scored 69 polymorphic characters which were shared by two or more taxa. All of the results yielded a data matrix that is available upon request. Our PHYLIP version could not be used due to the

large number of recorded characters. Using the unweighted pair group with mathematical average method (24) in the MEGA program, we obtained the tree shown in Fig. 6. Similar results were obtained by using the neighbor-joining method (data not shown). Figure 7 shows a phylogenetic tree generated by using the heuristic search option in PAUP, which was extremely similar to the consensus tree obtained by distance analysis except that strains Son2110 and NY1-86 branched differently in the two trees. As in the PFGE data analysis, all of the trees generated from the AP-PCR data analysis either by maximum parsimony or by genetic distance divided the 61 taxa into three major phylogenetic branches (branches I, II and III).

A total of 15 of the 33 European strains were grouped in PFGE polymorphism groups M, O, and R, which were closely clustered in all of the trees. In a majority of the trees generated by the distance analysis and by the parsimony analysis, these three groups appeared on the same phylogenetic branch as groups N and S. Group S was a mixed group containing both European and American strains; the PFGE patterns of these strains were identical, although the AP-PCR fingerprints were heterogeneous. The average pairwise genetic distances determined by the AP-PCR between strains belonging to groups S and O were 0.112 ($\sigma = 0.053$) and 0.136 ($\sigma = 0.055$), respectively, whereas the distance between groups S and O was 0.206. The single strain in group N was closely related to group S strains. The European strains in group Q were located in a heterogeneous cluster together with American strains, although the distances reflected by the branch lengths were large.

American strains were scattered on all of the main phylogenetic branches and were separated by large genetic distances. Only one representative of group B and one representative of J revealed the deepest branch. Particularly noteworthy is the closeness between American type strain B31 and some European strains in mixed group S. Similarly, European groups P and Q had American groups as their nearest neighbors.

To assess the range of genetic variation within *B. burgdorferi* sensu stricto, we used genetic markers which are widespread on the genome. A good correlation was observed between the two approaches used, as revealed by different phylogenetic computing methods, although AP-PCR identified more polymorphism groups than PFGE identified. We concluded two things from our study. First, greater polymorphism was observed among American strains than among European strains. Second, some American and European strains are closely related, which is consistent with the hypothesis that European

FIG. 5. Strict consensus of two trees obtained by maximum-parsimony analysis. The tree was generated from a reduced distance matrix for 20 taxa, each of which represented a PFGE polymorphism group, and was solved by the heuristics method contained within the PAUP package. The European strains are underlined. The number of isolates in each group is indicated in brackets.

FIG. 6. Unweighted pair group with mathematical average rooted tree obtained with MEGA software from AP-PCR data matrix. The European strains are underlined.

and American strains evolved from a very recent common ancestor. The different groups of American strains are more heterogeneous since they belong to distinct lineages. The deeper branching of these strains on distance trees is consistent with the hypothesis that American strains have been evolving for a longer period of time than European strains and could be ancestors of European strains.

However, to confirm the genetic structure of the population within the species, an alternative strategy would be to analyze strains with methods involving a single gene locus. The results of an analysis of different sequences suggest that strains isolated on each side of the Atlantic Ocean are highly related one another. American strains B31^T (T = type strain) and N40 and European strain ZS7 exhibited 99 to 100% *ospA* sequence identity (16, 32, 34). American strains B31^T (accession number X15661) and HB19 (accession number X75200) and European strain GeHo (accession number X15660) exhibited more than

FIG. 7. 50% majority rule tree obtained from 100 shortest trees by parsimony analysis. The tree was based on an analysis of 4,278 characters (62 strains $\times$ 69 characters) obtained by AP-PCR and was solved by the heuristics method contained within the PAUP package. The European strains are underlined.

99% *fla* sequence identity. Because of lateral transfer (19), many sequences of the variable *ospC* gene are available from data banks. A comparison of *ospC* sequences leads to the same conclusions as the analysis of PFGE and AP-PCR results. Livey et al. (19) detected more *ospC* types among American *B. burgdorferi* sensu stricto strains than among *B. burgdorferi* sensu stricto strains from Europe. It is interesting that strains classified in *ospC* restriction fragment length polymorphism type 2 by these authors all belong to unique PFGE polymorphism group S and are closely related as determined by AP-PCR. As observed for the whole genome, a striking similarity was reported for the *ospC* genes from strains isolated from distant geographic areas. For example, European strains IP2 (accession number L42887) and PKa (accession number X69589) and American strains B31^T (accession number X69596) and 26816 (18) exhibit 100% *ospC* sequence identity. These strains fell into the same PFGE polymorphism group. Similarly, American strain BUR (accession number X84765) exhibited 98.7 and 96% sequence identity with European strains ZS7 (accession number L42868) and DK7 (accession number X73625), respectively (15, 19, 27, 28). Our results for the whole genomes of *B. burgdorferi* sensu stricto isolates and the analysis of gene sequence data from data banks lead to the conclusion that European strains are closely related to and therefore very recently diverged from a few American clones. In addition, in America, *B. burgdorferi* sensu stricto has been evolving for enough time to diverge into at least two genomospecies related to one another at borderline species level (22). This is not the case in Europe. *B. burgdorferi* sensu stricto has not been found in Asia. This leads us to suggest anteriority of American strains, some of which could have migrated to Europe very recently. Other epidemiological features favor of this hypothesis. The main symptoms of Lyme borreliosis initially reported in Europe were neurological manifestations which are now known to be preferentially due to *B. garinii* and late cutaneous lesions preferentially due to *B. afzelii* (1, 2, 22, 29). Arthritis associated with *B. burgdorferi* sensu stricto is still rare in Europe, was described only recently, and is predominant in western Europe, and these facts could reflect a slow extension of a recently imported *B. burgdorferi* sensu stricto population from America. An extensive study of relatively conserved genes, such as *ospA* and *fla*, from a great number of strains from both continents could help confirm the hypotheses described above.

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