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Comparative Analysis of Genetic Variability among *Borrelia burgdorferi* Isolates from Europe and the United States by Restriction Enzyme Analysis, Gene Restriction Fragment Length Polymorphism, and Pulsed-Field Gel Electrophoresis

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The genomes of 62 North American and European *Borrelia burgdorferi* isolates were examined by restriction endonuclease analysis (REA), gene probe restriction fragment length polymorphism, and pulsed-field gel electrophoresis (PFGE). Hybridization of restriction fragments with the immunologically relevant 83-kDa antigen gene revealed polymorphisms and divided the isolates into three major groups. Group I included all but two of the American isolates and some of the European isolates. One of two Californian isolates (DN 127) and one *Ixodes dammini* isolate from New York (strain 25015), previously described as atypical, were distinct from the isolates in the three groups. Plasmid profile analysis and REA, the method with the highest level of discrimination, revealed extensive heterogeneity among isolates of the same major group. Our study demonstrates the usefulness of the 83-kDa antigen gene probe for dividing the isolates into major genogroups, whereas REA and plasmid profile analysis allow for a distinction of individual strains within these groups.

Since the first isolation of the causative agent of Lyme disease, *Borrelia burgdorferi* (17), more than 10 years ago, Lyme disease has been identified in many parts of the world (40). It became the most prevalent tick-associated disease in the United States (42) and was declared nationally reportable in 1991 (18). Although Lyme disease has been reported in 46 states, the three main foci are the Northeast (Massachusetts through Pennsylvania), the northern Midwest (Minnesota and Wisconsin), and the Pacific Northwest (parts of California, Oregon, and Washington) (40). *B. burgdorferi* has been cultured from a variety of tick vectors and vertebrate hosts, including humans. The tick vectors vary according to geographical regions and are primarily *Ixodes ricinus* in Europe and *Ixodes pacificus* in California (40). The main tick vector in the eastern and midwestern United States is the deer tick, *Ixodes dammini* (40), which was recently determined not to be a separate species from *Ixodes scapularis* (34). In this report it will therefore be referred to as *I. scapularis*.

Investigations into the genotypic and phenotypic characteristics and variations of *B. burgdorferi* isolates from different geographical and biological origins were prompted by variations in the clinical presentations of patients with Lyme disease (1, 10, 12, 14, 16, 27, 41, 45–47). On the basis of DNA-DNA reassociation experiments and 16S rRNA studies (10, 30, 37), three genomic species are delineated within what was once thought to be the homogeneous *B. burgdorferi* species group. Genospecies I corresponds to *B. burgdorferi* sensu stricto. Genospecies II has been named *Borrelia garinii*, and genospecies III is referred to as the VS461 group. Both new species contain isolates from Europe and Japan (10, 30).

To develop and improve diagnostic and preventive tools, it is imperative to have a good understanding of the heterogeneity prevalent within a pathogenic bacterial species. The objective of our study was to conduct a comparative genetic analysis of a substantial number of *B. burgdorferi* isolates from the United States and Europe in order to test whether genotypic differences between isolates might be associated with geographical location and variation in clinical manifestations observed in patients with Lyme borreliosis. Special emphasis was placed on a chromosomally conserved antigen gene, expressing an 83-kDa protein, that is highly specific for *B. burgdorferi*, as determined by immunoblotting (28, 36). The genotypes of 62 *B. burgdorferi* strains were examined by three methods with increasing levels of discrimination; gene restriction fragment length polymorphism (RFLP), plasmid profile (PP) analysis, and restriction endonuclease analysis (REA). We compared the results of the present study with results of a previous investigation, in which 29 *B. burgdorferi* isolates from California were analyzed by the same methods (48), and we also related the genotypic groupings obtained through our assays to those of different studies done in other laboratories.

MATERIALS AND METHODS

Bacterial isolates and culture conditions. The *Borrelia* strains used in the present study are listed in Table 1. BUR and ARC as well as BK1 and BK2 were used at a low passage number (less than 10). The exact passage number was unknown for the remaining isolates; it was, however,

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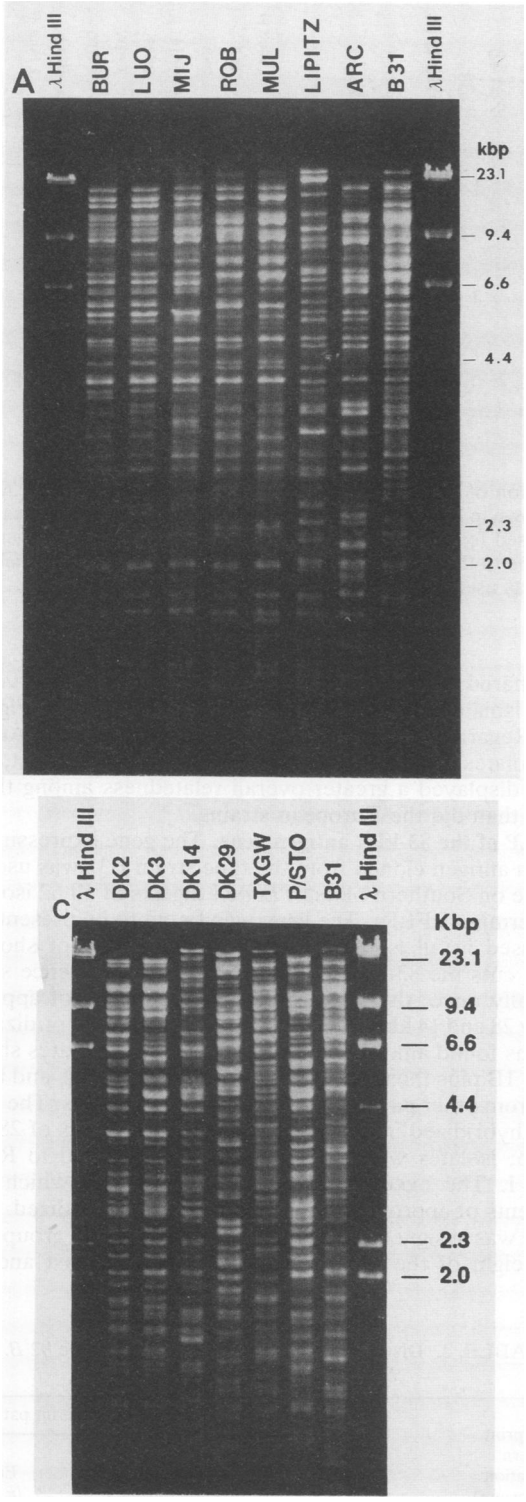
TABLE 1. Origins and RFLP profiles of *B. burgdorferi* isolates used in the study

Strain	RFLP	Origin		
		Host	Geographic	Reference or source
B31	I	*I. dammini*	New York	11
20001	I	*I. ricinus*	France	3
IP2	I	Human CSF[a]	France	9
P/Ka	I	Human CSF	Germany	38
P/Bi	V	Human CSF	Germany	38
DK29	V	Human skin	Denmark	K. Hansen
K48	V	*I. ricinus*	Czechoslovakia	K. Kmety
20047	V	*I. ricinus*	France	3
G25	V	*I. ricinus*	Sweden	43
P/Sto	VI	Human skin	Germany	38
DK2	VI	Human skin	Denmark	K. Hansen
DK3	VI	Human skin	Denmark	K. Hansen
DK14	VI	Human skin	Denmark	K. Hansen
ARC	I	Human skin	New York	A. McDonald
BUR	I	Human skin	New York	A. McDonald
LIPITZ	I	Human skin	New York	A. McDonald
LUO	I	Human skin	New York	A. McDonald
MIJ	I	Human skin	New York	A. McDonald
MUL	I	Human skin	New York	A. McDonald
ROB	I	Human skin	New York	A. McDonald
TXGW	I	Human skin	Texas	J. Rawlings
245	I	Human blood	Connecticut	A. Barbour
2591	I	*Peromyscus leucopus*	Connecticut	J. Anderson
21305	I	*P. leucopus*	Connecticut	J. Anderson
26105	I	*I. dammini*	Connecticut	J. Anderson
26278	I	*I. dammini*	Connecticut	J. Anderson
26375	I	*I. dammini*	Connecticut	J. Anderson
26815	I	Chipmunk	Connecticut	J. Anderson
27579	I	*I. dammini*	Connecticut	J. Anderson
27985	I	*I. dammini*	Connecticut	J. Anderson
29592	I	*I. dammini*	Connecticut	J. Anderson
29805	I	*I. dammini*	Connecticut	J. Anderson
29850	I	*I. dammini*	Connecticut	J. Anderson
29968	I	*I. dammini*	Connecticut	J. Anderson
30655	I	*P. leucopus*	Connecticut	7
30667	I	*P. leucopus*	Connecticut	7
30757	I	*P. leucopus*	Connecticut	J. Anderson
35653	I	*P. leucopus*	Connecticut	J. Anderson
36212	I	*P. leucopus*	Connecticut	7
28354	I	*I. dammini*	Maryland	J. Anderson
BK1	I	*I. dammini*	Massachusetts	B. Kimsey
BK2	I	*I. dammini*	Massachusetts	B. Kimsey
19535	I	*P. leucopus*	New York	J. Anderson
25015	VII	*I. dammini*	New York	J. Anderson
27982	I	*I. dammini*	Pennsylvania	5
28018	I	*I. dammini*	Pennsylvania	5
28019	I	*I. dammini*	Pennsylvania	5
28029	I	*I. dammini*	Pennsylvania	5
28032	I	*I. dammini*	Pennsylvania	5
28128	I	*I. dammini*	Pennsylvania	5
28132	I	*I. dammini*	Pennsylvania	5
28136	I	*I. dammini*	Pennsylvania	5
28143	I	*I. dammini*	Pennsylvania	5
28691	I	*I. dammini*	Pennsylvania	5
26816	I	*Microtus* sp.	Rhode Island	J. Anderson
21343	I	*P. leucopus*	Wisconsin	4
21528	I	*I. dammini*	Wisconsin	4
21594	I	*I. dammini*	Wisconsin	4
21596	I	*I. dammini*	Wisconsin	4
21645	I	*I. dammini*	Wisconsin	4
DN127	III	*I. pacificus*	California	46
SON188	I	*I. pacificus*	California	46

[a] CSF, cerebrospinal fluid.



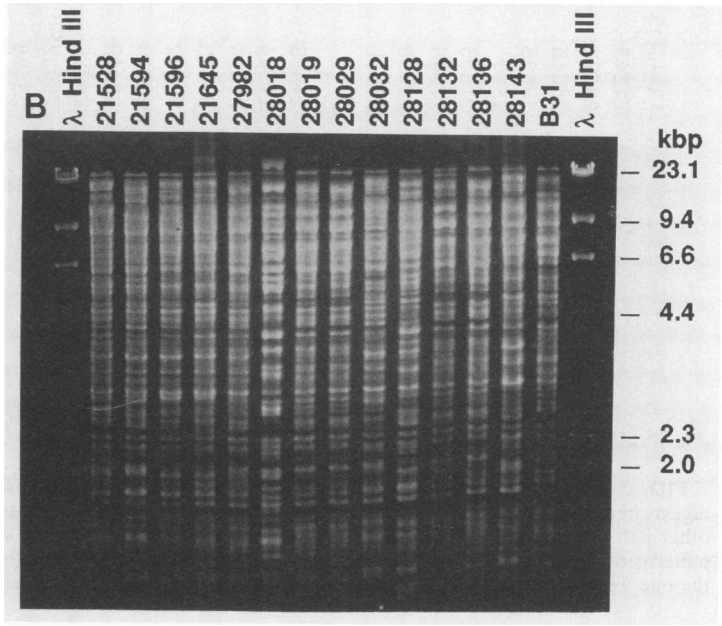


FIG. 1. Fingerprint patterns derived from REA of *Eco*RI-digested chromosomal DNAs of 28 *B. burgdorferi* isolates from the United States and Europe. Shown are photographs of ethidium bromide-stained 1% agarose gels. The electrophoresis conditions constituted 14 h and 30 min at 60 V in 0.5× TBE buffer (1× TBE buffer is 89 mM Tris-borate, 89 mM boric acid, and 2 mM EDTA). Approximately 2.5 μg of DNA was loaded per well. Bacteriophage lambda DNA digested with *Hin*dIII was used as a size marker and is indicated on the right sides of the three panels (in kilobase pairs). (A) Chromosomal fingerprint patterns of seven *B. burgdorferi* strains isolated from the skin of patients with Lyme disease in the state of New York. (B) Chromosomal fingerprint patterns of tick isolates from the central and eastern United States. Displayed in the first four lanes are *I. dammini* isolates from Fort McCoy, Wis.; the remaining lanes represent strains isolated from *I. dammini* ticks collected in Huntington Valley, Pa. (C) Chromosomal fingerprint patterns of four Danish skin isolates (DK2, DK3, DK14, and DK29), one skin isolate from Texas (TXGW), and one skin isolate from Germany (P/Sto).

greater than 10 in all cases. All isolates were maintained in modified Barbour-Stoenner-Kelly (BSK) medium at 33°C (11).

Chromosomal DNA enrichment and REA. Previously described methods for extraction and purification of *Borrelia* DNA were used (48). The enzymes were chosen on the basis of information presented in a previous study (26).

Southern blotting and DNA hybridization. Electrophoresed DNA restriction fragments were transferred to a nylon membrane and hybridized to the cloned 83-kDa antigen gene of *B. burgdorferi* B31 (28) as described previously (48).

Pulsed-field gel electrophoresis. *Borrelia* cells were embedded in plugs of 0.5% low-melting-temperature agarose as described previously (48). The double inhomogeneous electrode array was used, and the point electrodes were set as described previously (26). The gels were run with a switching time of 1 s, resulting in an optimal resolution range of 10 to 40 kbp.

RESULTS

REA. Sixty-two *B. burgdorferi* isolates obtained from various biological and geographical sources in Europe and the United States were examined. Figures 1A through C demonstrate the chromosomal fingerprint patterns of 27 of these isolates when their chromosomes were digested with *Eco*RI. These representative isolates were selected to dem-

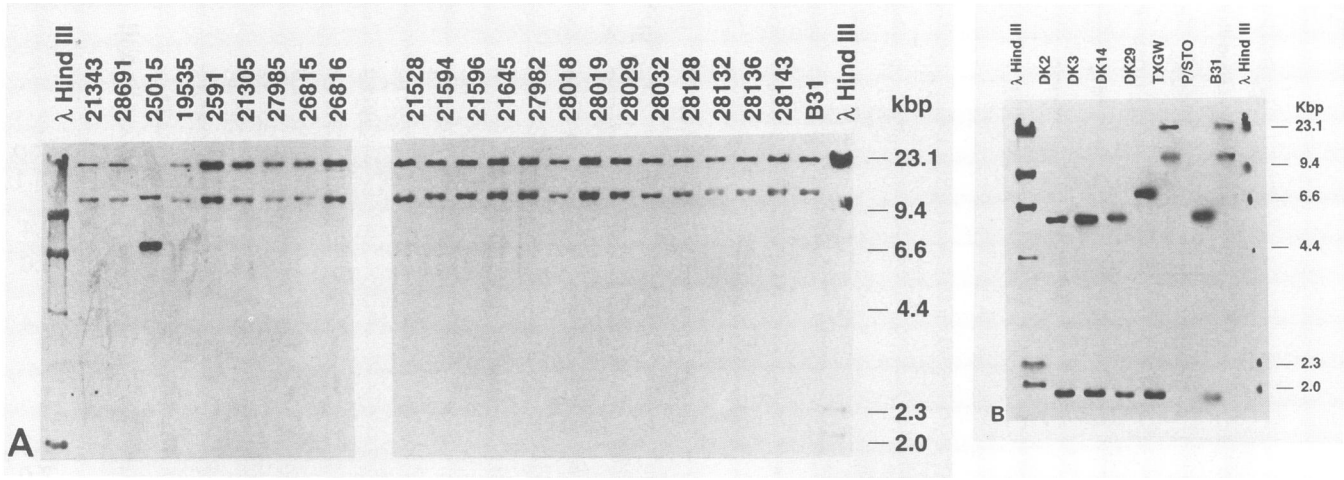


FIG. 2. (A) Fingerprint patterns obtained through Southern blot hybridization of the 83-kDa antigen gene to the chromosomal *Eco*RI digests of the tick isolates from central and eastern parts of the United States shown in Fig. 1B and of six mammal and three tick isolates from other parts of the eastern United States. Enhanced chemiluminescence was used for labeling and detection of the probe. (B) Fingerprint patterns obtained through Southern blot hybridization of the 83-kDa antigen gene to the chromosomal *Eco*RI digests of the four Danish and the one Texas skin isolates shown in Fig. 1C. Enhanced chemiluminescence was used for labeling and detection of the probe.

onstrate the similarities and differences of the total number of isolates studied. They exemplify the various sources of the isolates (ticks, rodents, humans, etc.) and their geographical distributions. B31 was used as the reference strain on each gel. Figure 1A illustrates the *Eco*RI restriction fragment configuration of seven isolates cultured from the skin of Lyme disease patients in New York State. BUR and LUO are very similar to each other, differing in only a few bands in the proximity of the ranges of 8 to 8.5 kbp and 3.6 to 3.8 kbp, respectively. ROB and MUL appeared to be identical and also shared similarity with MIJ. LIPITZ and ARC share only a few bands with the other isolates from human skin and otherwise demonstrated unique banding patterns. None of these isolates from human skin had an REA pattern identical to that of the reference strain.

The *Eco*RI restriction profiles of isolates cultured from *I. scapularis* ticks collected in Wisconsin and Pennsylvania are shown in Fig. 1B. Visual examination of Fig. 1B reveals the high degree of similarity between most of the isolates; strain 28018, however, displayed a very distinctive banding pattern.

A high degree of heterogeneity was found among isolates from Europe, illustrated by the *Eco*RI digestion patterns of four human skin isolates from Denmark and one human skin isolate from Germany (Fig. 1C). The two Danish isolates DK2 and DK3 showed related yet still distinctive patterns. The others demonstrated very distinct REA profiles. None of the isolates showed an electrophoretic fragment configuration similar to that of the reference strain B31. A human cerebrospinal fluid isolate from Germany (P/Bi) and an *I. ricinus* isolate from Sweden (G25) revealed identical REA patterns (data not shown). Chromosomal digests of all isolates with *Hin*dIII, *Hha*I, and *Cla*I confirmed the similarities and differences observed with the *Eco*RI digest.

The chromosomal DNAs of one tick and two rodent isolates from Connecticut (26278, 30667, and 36212) and of four tick isolates from Pennsylvania (28019, 28029, 28132, and 28136) were not cut when they were digested with the enzyme *Hha*I. Correspondingly, the DNA of the Californian tick isolate SON188 could not be cut with *Hin*dIII.

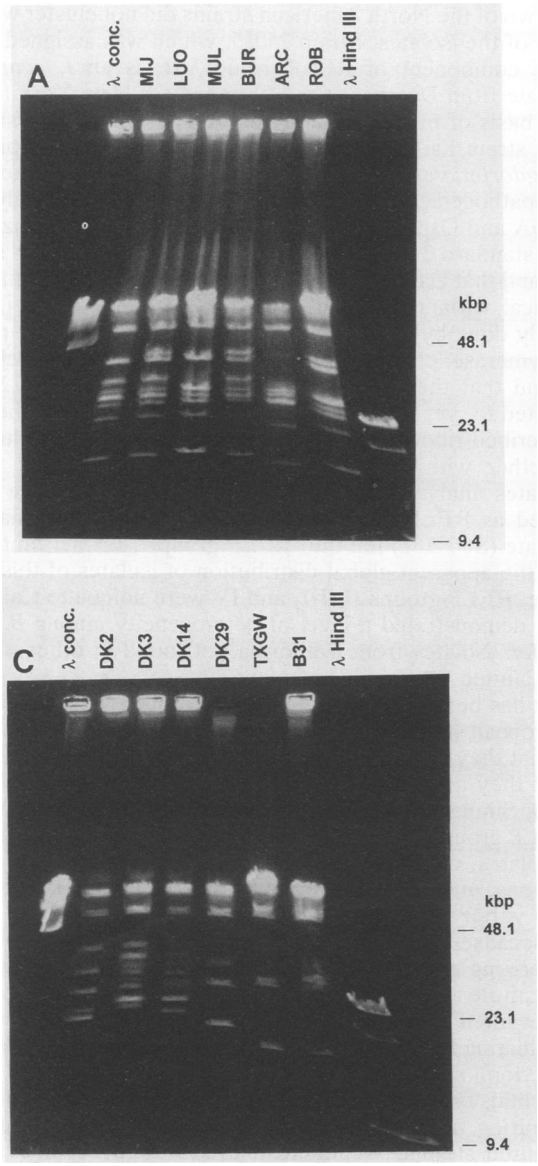
The REA profiles of the 35 isolates not shown in Fig. 1A to C shared the level of similarity or difference observed in the 27 isolates for which the results are displayed in Fig. 1A to C. Regardless of the biological source, the North American isolates from the Northeast, the upper Midwest, and Texas displayed a greater overall relatedness among themselves than did the European strains.

RFLP of the 83-kDa antigen gene. The gene expressing an 83-kDa antigen cloned from the type strain B31 was used as a probe on Southern blots of *Eco*RI digests of all 62 isolates to determine RFLPs. The gene was found to be present and expressed in all isolates tested to date (data not shown). *Eco*RI cuts the 83-kDa antigen gene of the reference strain internally once, thus generating two fragments of approximately 28 and 14 kbp. Figure 2A represents the hybridization patterns found among the same *I. scapularis* isolates shown in Fig. 1B plus those of isolates from six mammals and three ticks from other parts of the eastern United States. The gene probe hybridized in all but one case to two bands of 28 and 14 kbp; isolates with this pattern were assigned to RFLP group I. The exception was strain 25015, to which two fragments of approximately 15 and 6.8 kbp hybridized. This isolate was assigned its own unique RFLP group, group VII. Forty-eight of the 49 isolates from the Northeast and the

TABLE 2. Distribution of RFLP groupings in the 92 *B. burgdorferi* isolates tested

Fingerprint pattern designation (RFLP group)	No. of isolates showing each fingerprint pattern		
	California (n = 31)[a]	Central and eastern United States (n = 48)	Europe (n = 12)
I	11	47	3
II	5	0	0
III	11	0	0
IV	4	0	0
V	0	0	5
VI	0	0	4
VII	0	1	0

[a] Numbers in parentheses refer to the total number of isolates tested.



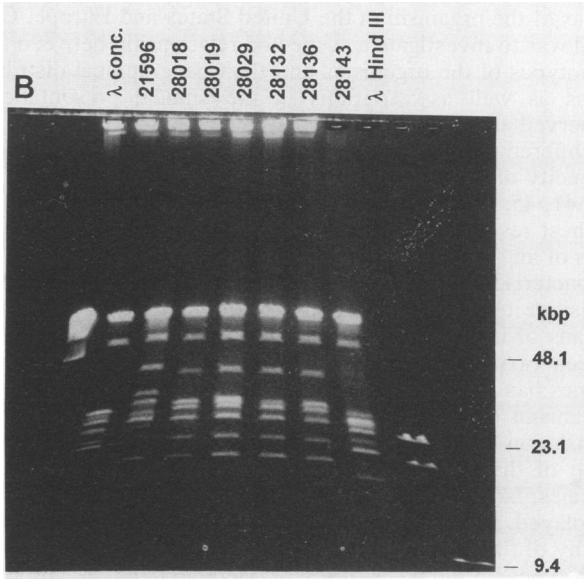


FIG. 3. Pulsed-field gel electrophoresis demonstrating the PPs of *B. burgdorferi* isolates determined with the double inhomogeneous electrode array. The cells were lysed in situ, and the DNA was analyzed on a 1% agarose gel for 24 h at 300 V with a switch time of 1 s. Size markers were bacteriophage lambda multimers (λ-conc.) and *Hin*dIII-digested bacteriophage lambda DNA (in kilobase pairs). (A) PPs of six of the New York human skin isolates. (B) PPs of some of the *I. dammini* isolates from Wisconsin (21596) and Pennsylvania (all remaining lanes). (C) PPs of the four Danish skin isolates, and the Texas skin isolate *B. burgdorferi* B31, the reference strain, was also analyzed on the gel shown in panel C.

Midwest (including Texas) displayed the RFLP group I hybridization pattern. A different and more heterogeneous fingerprint pattern was found among the European isolates, as demonstrated in Fig. 2B, which displays the autoradiograph of the Southern blotted gel shown in Fig. 1C. Although some European isolates exhibited an RFLP group I hybridization pattern, the majority revealed either one of two additional distinct fingerprint patterns, characterized by hybridizing fragments of 7.6 and 1.9 kbp (DK29, P/Bi, K48, 20047, and G25) and fragments of 6 and 1.9 kbp (DK2, DK3, DK14, and P/Sto), respectively. Isolates with these patterns were designated RFLP groups V and VI, respectively. Isolates of RFLP groups II, III, and IV were assigned to fingerprint patterns unique to the Californian isolates (48). The RFLP groupings are listed in Table 1. The clustering of all strains, including that of the 29 Californian isolates examined by the same method as was used in the previous study (48), is displayed in Table 2.

PP analysis. All *B. burgdorferi* isolates were analyzed for their plasmid contents. Our electrophoresis conditions revealed a range of between 3 and 10 plasmids, with the majority of isolates containing 7 or 8 extrachromosomal pieces of DNA. The plasmid sizes ranged from 16 to approximately 65 kbp on the basis of linear DNA sizing. A plasmid of approximately 65 kbp is sized at 49 kbp when using conventional agarose gel electrophoresis (13, 25) and was shown to encode the outer surface proteins (Osps) A and B in several *B. burgdorferi* isolates (13). Strain 25015 was the only isolate in the present study that did not contain a plasmid of this size (data not shown). Figure 3A shows the PPs of six of the New York human skin isolates. BUR and LUO appeared to be almost identical in their plasmid number and sizes. MIJ, ROB, and MUL were similar to each other and to LUO and BUR, differing in the absence of a single plasmid for MIJ and MUL and two plasmids for ROB. ARC had the most unique PP of these isolates, containing only six plasmids, in spite of being a low-passage-number strain. Four of the North American *B. burgdorferi* isolates from ticks shown in Fig. 3B (28019, 28029, 28132, and 28136) displayed identical PPs. These isolates also had identical REA patterns (Fig. 1B). Strain 28018, which showed a distinct REA profile, also displayed a distinct PP. The heterogeneity found among the PPs of the 12 European strains correlated with the diversity of their chromosomal fingerprint patterns (Fig. 3C). Only G25 and P/Bi had identical PPs.

DISCUSSION

In the most extensive study completed to date, we examined 62 *B. burgdorferi* isolates from the three main focal

areas of the organism in the United States and Europe. Our goal was to investigate a suspected relationship between the genotypes of the organisms and their geographical distributions as well as variations in the clinical presentations observed among patients with Lyme disease.

Different approaches have been used to survey the heterogeneity of the *B. burgdorferi* species group (1, 10, 30, 31, 37, 41, 45, 46). Actual DNA sequence analysis renders the highest resolution when investigating the genetic composition of an organism (29). In analyzing intraspecies variation in bacteria, REA assays, which are much simpler yet highly sensitive to small changes in DNA organization, provide a means of indirectly estimating nucleotide substitutions between two sequences (19, 29). REA of bacterial chromosomes has proven to be a reliable method of characterizing organisms (26, 27, 35). In our study, we demonstrated that restriction endonuclease fingerprinting provides a high degree of discrimination between strains within a *Borrelia* species. Isolates from the American Northeast and Midwest displayed a relatively high level of interstrain similarity. Many of them, however, could still be classified as distinct genotypes on the basis of their collective banding patterns.

The DNAs of seven North American *B. burgdorferi* isolates (26278, 30667, 36212, 28019, 28029, 28132, and 28136) were not digested by the endonuclease *Hha*I, which recognizes the sequence GCGC. An internal or external C5-methylcytosine or the conversion of both cytosines into hydroxymethylcytosines prevents the enzyme from cutting the DNA (33). *Hin*dIII did not cut the DNA of the Californian isolate SON188. This restriction enzyme recognizes the hexanucleotide sequence AAGCTT and does not cleave in the case of a methylated cytosine residue nor in the case of methylation at the external adenine residue (33). Hughes and Johnson (24) reported that 3 of 22 *B. burgdorferi* strains that they examined contained an adenine methylation system of the *dam* type. Methylases with possibly similar functions yet different specificities from the well-described *Escherichia coli dam* and *dcm* enzymes have been reported in *Bacillus subtilis* and *Staphylococcus aureus* (20). Our results suggest that at least one additional methylation system with a specificity different from that of the *dam* methylase occurs among *B. burgdorferi* strains.

Hybridization of either randomly cloned genomic fragments (19, 35, 41) or selected cloned genes (35, 44, 45) to enzyme-restricted chromosomal DNA can also render unique and reproducible DNA fingerprint patterns. Although this technique is more labor-intensive, it reduces the complexity of the REA fingerprint patterns, thereby facilitating comparisons between strains. The 83-kDa antigen gene (28) was used as a probe. Sixty of the North American and European isolates clustered into three major genomic groups, and there were only two divergent isolates (25015 and DN127). A subset of these isolates was examined in independent studies; those investigators found genetic clusterings analogous to the ones described here (10, 16, 46). The largest RFLP group (group I), containing all but one of the isolates from the American Northeast and Midwest (including Texas) as well as three of the European isolates and the Californian isolate SON188, corresponds to what is now considered *B. burgdorferi* sensu stricto. Our RFLP group V contained three isolates (20047, G25, and P/Bi) which were previously determined to belong to a separate species (10, 16, 46). European isolates falling into RFLP group VI have, to our knowledge, not been analyzed in any other laboratory. It is possible that these isolates are part of the newly described genospecies named the VS461 group (10).

Two of the North American strains did not cluster with the rest of the isolates. Strain 25015, which was assigned as the sole component of RFLP group VII, is an *I. scapularis* isolate from Dutchess County in upstate New York (6). On the basis of protein banding patterns and infection studies, this strain had previously been reported as an atypical *B. burgdorferi* variant. It was demonstrated to be infectious but nonpathogenic in laboratory rats and mice (2). Analysis of OspA and OspB revealed that they were larger in size than the standard 31- and 34-kDa proteins found in other strains (2) and that OspA of 25015 differed from the OspA of a more typical variant (N40) in 40 of its 273 amino acids (22). A study done by Welsh et al. (46) using an arbitrarily primed polymerase chain reaction as a fingerprinting technique found that the Californian *I. pacificus* isolate DN127 was related to, yet somewhat different from, isolates of the three described ribotypes. We found that strain DN127 clustered together with 10 of 29 isolates from Californian rodent isolates analyzed in a previous study into what we designated as RFLP group III (48). The other Californian tick isolate (SON188) fell into RFLP group I, further emphasizing the apparent global distribution of isolates of this genotype. RFLP groups II, III, and IV were unique to California and demonstrated a level of heterogeneity among *B. burgdorferi* isolates from California not found in other parts of the United States.

It has been suggested that the reason that the two unique European genomic groups are not found in the United States is that they cannot efficiently use *I. scapularis* as a vector or that they are restricted by the mammalian host population (46). Similar constraints could be limiting the three unique RFLP groups found in California. Conversely, RFLP group I isolates, with their wide geographical and biological distributions, undoubtedly seem highly flexible in their habitation of a variety of vectors and hosts.

Because PP analysis has been used successfully for characterizing a variety of bacterial species (32) and plasmids constitute a significant portion of the *B. burgdorferi* genome, we wanted to assess the use of plasmid profiling as a tool for evaluating heterogeneity within this species. Pulsed-field gel electrophoresis, which is ideal for separating the linear plasmids typical of *Borrelia* species, is an efficient procedure requiring no DNA extraction and only small amounts of cultured strains. We found that REA and PP analysis are comparable in their efficiencies in discriminating *B. burgdorferi*. Furthermore, we found that isolates of the same RFLP group which had identical or very similar REA profiles often displayed identical or very similar PPs. Reports of a linear chromosome in *B. burgdorferi* have led to suggestions that the linear plasmids may in fact be minichromosomes (15, 21). Telomere-like structures discovered on the ends of two of these linear plasmids lend credibility to this proposal (23). Our finding that linear plasmid size and number correlate with an overall genotype also suggests a greater stability for these extrachromosomal molecules than normally observed with plasmids in other bacteria. Although a rare event, *B. burgdorferi* has been shown to lose certain plasmids spontaneously over the course of serial in vitro passaging (39), and in the present study, we reported the loss of the plasmid encoding OspA and OspB from strain 25015.

In assessing the genetic heterogeneity among 62 isolates of the *B. burgdorferi* species group, we found the most pronounced chromosomal and plasmid heterogeneities among European and Californian strains. Seventeen isolates from humans were analyzed in the present study. Several of the isolates from skin demonstrated identical RFLPs and iden-

tical or very similar REA profiles and PPs. However, other isolates from skin and all of the isolates from cerebrospinal fluid demonstrated unique REA profiles and PPs. A clearer link was observed between genotype and geographical distribution and/or host. The majority of North American isolates clustered into RFLP group I. The REA patterns and plasmid profiles of the *I. scapularis* isolates demonstrated considerable similarities to each other. The geographical distribution of RFLP groups II, III, and IV appears restricted to Californian isolates. RFLP groups V and VI were localized to Europe. The speculation that *B. burgdorferi* evolved in Europe because of greater genetic diversity (45) seems less plausible on the basis of the genetic heterogeneity of this organism in California. Although there is some evidence that human skin isolates, for example, may share similarities, a larger number of isolates from humans needs to be examined to conclusively assess the proposed relationship between clinical manifestations and genotype (8, 12, 14, 26, 47).

The extent of intraspecies heterogeneity can have major implications for the development of diagnostic assays and vaccines. Our results confirm the fact that extensive genetic heterogeneity exists among *B. burgdorferi* isolates and that the degree of heterogeneity is greater in Europe and California than in the central and eastern United States. Furthermore, it demonstrates a high level of genetic variation among isolates from identical organ systems of human patients. The genogroup divisions resulting from our 83-kDa antigen gene fingerprinting correlate with data obtained by other typing methods. Our results confirm the usefulness of REA and PP analysis as highly discriminating techniques that are useful for the subtyping of *B. burgdorferi* isolates within larger genogroups established by methods of lesser discriminatory power.

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