Most bacterial pathogens comprise a variety of strains in various proportions. For *Borrelia burgdorferi*, an agent of Lyme borreliosis, strains differ in their reservoir host preferences (1), propensities to disseminate in humans (2,3), and prevalences in ticks by geographic area (4,5). Strain identification of *B. burgdorferi* now is predominantly based on DNA sequences of either of 2 genetic loci: 1) the plasmid-borne, highly polymorphic outer surface protein C gene and *rrs*-rrlA intergenic spacer from extracts of *Ixodes* spp. ticks in 3 US regions showed linkage disequilibrium between the 2 loci within a region but not consistently between regions.

*Geographic Differences in Genetic Locus Linkages for* *Borrelia burgdorferi*

*Borrelia burgdorferi* genotype in the northeastern United States is associated with Lyme borreliosis severity. Analysis of DNA sequences of the outer surface protein C gene and *rrs*-rrlA intergenic spacer from extracts of *Ixodes* spp. ticks in 3 US regions showed linkage disequilibrium between the 2 loci within a region but not consistently between regions.

For 741 *Ixodes* ticks from northeastern and north-central United States or from northern California, 1 *ospC* allele was identified and sequenced. In the remaining samples, we found a mixture of strains or evidence of >2 *ospC* and/or >2 IGS sequences (9). In 678 (91%) of the 741 samples with a single *ospC*, the allele could be matched with particular IGS1 (Table). We identified 9 unique *ospC* sequences: Fc, Ob, Ub, A3, B3, C3, D3, E3, and F3, all from the north-central United States. Alleles H3 and I3 of California were recently reported by Girard et al. (5). Of 32 codon-aligned *ospC* sequences, 6 pairs and 1 trio (Fa, Fb, and Fc) differed in sequence by <1% (Figure, panel A). Nine novel IGS1 sequences, numbered 24–31 and 33, were discovered in samples from which *ospC* alleles were determined.

When we confined analysis to samples from northeastern states, we confirmed linkage disequilibrium between *ospC* and IGS1 loci (7,10,14). However, when results from north-central states and California were included, a different picture emerged (Table, Figure, panel B). Most of the
ospC alleles showed concordance with the chromosomal loci; monophyletic MLST showed either the same ospC allele or a minor variant of it. However, in several instances, the ospC alleles were linked to different IGS1 sequences, different ospA sequences, and/or different MLST with internal nodes in common. We observed this linkage for ospC alleles A, G, Hb, and N. In the case of ospC Hb, the shared internal node was deep.

### Table. Linkages between ospC alleles and other loci in *Borrelia burgdorferi* strains

| ospC | IGS1 | Geographic region* | Representative cultured isolate or tick sample† | IGS1-ospC associations‡ | ospA | IGS2 | MLST§ |
|------|------|-------------------|---------------------------------|------------------------|------|------|-------|
| A    | 1    | 1, 2              | B31                             | 45/52                  | 1    | 1    | 1     |
| A    | 11   | 2                | 2206617                         | 4/4                    | 22   | 1    | 55    |
| A    | 10   | 3                | CA4, CA6                        | 14/18                  | 23   | 1    | 2     |
| Ba   | 3    | 1                | 64B, B373                       | 39/41                  | 3    | 1    | 7,58,59 |
| Ba   | 6    | 2                | 51405UT                         | 7/9                    | 14   | 1    | 30     |
| Bb   | 16   | 4                | Z57                            | –                      | 28   | –    | 20     |
| C    | 24   | 1                | JD1, BL515                      | 10/10                  | 8    | 5    | 11     |
| Da   | 5    | 1                | 516113                          | 13/14                  | 5    | 4    | 38     |
| Db   | 5    | 2                | 424404                          | 13/15                  | 18   | 7    | 51     |
| Db   | 19   | 3                | CA11.2A                         | 16/16                  | 27   | 4    | 70     |
| E    | 9    | 1, 2             | N40, B348                       | 17/19                  | 9    | 1    | 19     |
| Fa   | 17   | 1, 2, 3          | B156                            | 61/64                  | 3    | 4    | 8      |
| Fb   | 18   | 2                | M407                            | 14/19                  | 8    | 6    | –      |
| Fc   | 18   | 2                | 1469205                         | 7/8                    | 13   | 6    | 56     |
| G    | 26   | 1                | 72a, MR616                      | 10/11                  | 9    | 4    | 14     |
| G    | 22   | 2, 3             | 1468503                         | 9/10                   | 21   | 4    | 48,49  |
| Ha/Hb| 12   | 1                | B509/156a                       | 13/13                  | 2    | 2    | 4      |
| Hb   | 12   | 2                | 519014UT                        | 56/65                  | 11   | 2    | 32     |
| Hb   | 13   | 3                | CA92-0953                       | 20/20                  | 23   | 2    | 6      |
| Ia   | 7    | 1                | B500, B331                      | 12/16                  | 7    | 4    | 15,16  |
| Ia   | 7    | 2                | W91-23                          | 5/5                    | 11   | 4    | 71     |
| Ib   | 7    | 3                | CA92-1096                       | –                      | 30   | 4    | 17     |
| J    | 20   | 1, 2             | 11Bb                            | 3/5                    | 8    | 4    | 34     |
| K    | 2    | 1                | 297                             | 67/68                  | 2    | 2    | 3      |
| K    | 14   | 2                | 149901                          | 7/10                   | 31   | 2    | –      |
| L    | 14   | 2                | 47703UT                         | 23/25                  | 8    | 2    | 29     |
| M    | 6    | 1                | 29805                           | 4/4                    | 2    | 3    | 12     |
| M    | 6    | 2, 3             | CA92-1337                       | 16/16                  | 17   | 3    | 13     |
| N    | 4    | 1                | MR661, 500203                   | 41/41                  | 4    | 10   | 9,36   |
| N    | 23   | 2                | 51108                           | 8/10                   | 2    | 1    | 43     |
| Oa   | 27   | 1                | 501427                          | 1/1                    | –    | –    | 54     |
| Ob   | 6    | 2                | 2207807                         | 6/7                    | 2    | –    | –      |
| T    | 28   | 1                | 23509                           | 16/16                  | 8    | 4    | 37     |
| T    | 29   | 2                | 1476702                         | 10/11                  | 20   | 4    | 46     |
| Ua   | 8    | 1                | 94a, B485                       | 19/19                  | 8    | 4    | 18     |
| Ua   | 8    | 2                | 48802                           | 4/4                    | 16   | 4    | 47     |
| Ub   | 17   | 2                | 2207116                         | 4/4                    | 12   | 10   | –      |
| M    | 30   | 2                | 426905                          | 3/3                    | 8    | 9    | –      |
| A3   | 14   | 2                | 2206613                         | 6/6                    | 19   | 2    | –      |
| B3   | 23   | 1, 2             | 2250201                         | 3/3                    | 17   | 1    | 57     |
| C3   | 17   | 2                | 50202                           | 6/9                    | 15   | 5    | –      |
| D3   | 31   | 2                | 2150902                         | 1/1                    | –    | –    | –      |
| E3   | 20   | 2                | 2127701                         | 4/4                    | 8    | 8    | 52     |
| E3   | 21   | 3                | HRT25                           | 12/12                  | 24   | –    | –      |
| E3   | 5    | 3                | LMR28                           | 12/12                  | 25   | –    | –      |
| F3   | 5    | 2                | 1456802                         | 8/12                   | 8    | 4    | –      |
| H3   | 25   | 3                | CA8                             | 37/40                  | 26   | 4    | (72)   |
| I3   | 17   | 3                | CA11, CA12                      | 5/5                    | 27   | 4    | –      |

*Regions: 1, northeastern United States; 2, north-central United States; 3, northern California; 4, western Europe; osp, outer surface protein; IGS, intergenic spacer; MLST, multilocus sequence typing; –, MLST not determined.
†Number of tick extracts with the listed IGS1 locus (numerator)/number of extracts with the listed ospC allele (denominator).
‡MLST from (4,12) or this study (in parentheses).
We applied the Simpson index of diversity, as implemented by Hunter and Gaston (15), to the data in the Table to compare the discriminatory power (DP) of genotyping on the basis of a combination of *ospC* and IGS1 sequences with genotyping by 8-locus MLST (12). For double-locus typing, there were 43 types were found for 678 strains; DP value was 0.96. For MLST in this data set, 36 types were shown for 554 strains; DP was 0.95. In the study of Hoen et al. in which selection was made for geographic isolation, 37 types were distributed among 78 strains; DP was 0.97 (4).

**Conclusions**

Dependence on a single locus for typing may falsely identify different lineages as the same, especially when the samples come from different regions. Other loci may be as informative as *ospC* or IGS1, but the abundance of extant sequences for these loci justifies their continued use. Uncertainties about the linkage of *ospC* and IGS1 usually can be resolved by sequencing the *ospA* allele (Table). IGS2 provided little additional information in this study.

One interpretation of these findings is that lateral gene transfer of all or nearly all of an *ospC* gene has occurred between different genetic lineages. We previously had not detected recombination at the IGS1 locus on the chromo-
some (7), but there may be recombination at other chromosomal loci, as well as plasmid loci (6). Besides extending the understanding of the geographic structuring of the B. burgdorferi population, the results indicate that the ospC allele does not fully represent the complexity of B. burgdorferi lineages; thus, inferring phenotypes on the basis of this single locus should be made with caution.

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| Strain | ospC allele | IGS2 accession no. | IGS1 name | GQ463603 |
|--------|------------|--------------------|-----------|-----------|
| B31    | A          | AE000792           | 1A        | 1         |
| C4     | A          | EU377748           | 1A-684    | 10        |
| C5     | A          | EU377748           | 1A-684    | 10        |
| 2206617| A          | AE000792           | 1A-684/872| 11        |
| 64b    | Ba         | CP001422           | 3A        | 3         |
| 837    | Ba         | EU377779           | 3B        | 3         |
| 51405UT| Ba         | EU373625           | 6A        | 6         |
| ZS7    | Bb         | NC_011724          | 3D        | 16        |
| JD1    | Ca         | DG437462           | 5G        | 24        |
| BLS15  | Ca         | EU377774           | 5G        | 24        |
| OC4    | Da         | AF028863           | 5A        | 5         |
| 516113 | Da         | EU377521           | 5A        | 5         |
| 42404D | Db         | GQ478283           | 5A        | 5         |
| 11.2a  | Ca         | EU377521           | 5A-239    | 19        |
| N40    | E          | AY275221           | 9A        | 9         |
| B348    | E          | AF467875           | 9C        | 9C        |
| 990503 | Fa         | AY275225           | 4C        | 17        |
| B158    | Fa         | EU377776           | 4C        | 17        |
| M6107   | Fb         | EF357367           | 4C        | 18        |
| 1469205 | Fc        | GQ478285           | 4D        | 18        |
| MR616   | G          | EU377771           | 6B        | 26        |
| 72a     | G          | CP001375           | 6B        | 26        |
| 1468503 | G        | AY275223           | 5C        | 22        |
| B509    | Ha         | EU377781           | 2D        | 12        |
| 156a    | Hb         | CP001271           | 2D        | 12        |
| 519014UT| Hb         | EU375831           | 2D        | 12        |
| 519512  | Hb         | GQ478286           | 2D        | 12        |
| 401994  | K          | AY275214           | 2A        | 2         |
| 149901  | K          | AY275214           | 2E        | 14        |
| 47703UT | L          | EU375832           | 2E        | 14        |
| 29065   | M          | CP001550           | 6A        | 6         |
| 1472505 | Ila        | AF467874           | 7A        | 7         |
| 1469205 | Ila        | AF467874           | 7A        | 7         |
| 1472505 | Ila        | AY275219           | 7A        | 7         |
| 919-23  | Ipa        | CP001446           | 7A        | 7         |
| 1469205 | Ipa        | AY275223           | 5C        | 22        |
| 500203  | N          | AY275216           | 4A        | 4         |
| M518    | N          | EF573430           | 5E        | 23        |
| 51108   | N          | AY275216           | 5E        | 23        |
| 501427  | Oa         | FJ997281           | 6C        | 27        |
| 2207807 | Ob         | FJ997282           | 6D        | 6         |
| 23509   | T          | AY275222           | 8C        | 28        |
| 1476702 | T          | AY275222           | 8C-808    | 29        |
| 94a     | Ua         | CP001493           | 8A        | 8         |
| B485    | Ua         | EU377769           | 8A        | 8         |
| 48802   | Ua         | CP001493           | 8A        | 8         |
| 2207116 | Ua         | EU377769           | 8A        | 8         |
| 426905  | Ua         | GQ478287           | 8E        | 30        |
| 2206613 | A3         | EF592541           | 2E        | 14        |
| 2250201 | B3         | EF592542           | 5E        | 23        |
| 50202   | C3         | EF592543           | 4C        | 17        |
| 2150902 | D3         | EF592544           | New       | 31        |
| 1456802 | E3         | EF592545           | 5A        | 5         |
| C4      | H3         | FH3J2733           | 5A8       | 25        |
| CA11    | I3         | FH3J2734           | 4C        | 17        |
| CA12    | I3         | FH3J2734           | 4C        | 17        |

*Boldface indicates new accession number from this study.
†IGS1, ns=NA intergenic spacer region.
‡IGS2, mt=mt intergenic spacer.