test, df = 2), further supporting the conclusion that SA genes have accumulated in the experimental lines.

Replicate 2 of the experimentals is especially noteworthy because of the parity in fitness observed between wild-type red-eyed males and the orange-eyed males that expressed two recessive eye-pigment mutations that reduce visual acuity and thereby substantially reduce male mating success (8). I have used the net fitness assay in numerous other unrelated applications with the same or similar starting genetic backgrounds, and never have the wild-type flies performed so poorly against the double mutant standard, as was observed in replicate 2.

Owing to the lack of recombination in males, there was twice as much opportunity in the experimental lines for recombination to breakdown linkage disequilibrium (in the vicinity of the eye-color loci) that existed at the beginning of the experiments. This difference could elevate the fitness of orange-eyed males in the experimentals relative to controls if the chromosomal regions flanking the recessive markers were considerably less fit than the homologous regions flanking the wild-type alleles.

The observed pattern of genotype-specific fitness in replicate 1 of the experimentals might be explained by this interpretation, but not the pattern observed in replicate 2 (Table 1). The day 1 sex ratio assay (Fig. 2), however, demonstrates that SA genes accumulated in both experimental replicates; the correlation test (Fig. 2B) is statistically significant ($P < 0.05$) when each experimental replicate is individually compared to the two controls. The day 1 sex ratio assay controls for any confounding effects associated with differing rates of decay in initial linkage disequilibrium by comparing the fitness (viability and development time) of red-eyed males relative to females with identical levels of disequilibrium.

The first conclusion from these experiments is that we now have experimental support for the hypothesis that the chromosomal region proximate to a new sex-determining gene can act as a hot spot for the accumulation of genes that are detrimental to the homogametic sex. Such accumulation is vital to the operation of the current models proposed for the evolution of suppressed recombination between primitive sex chromosomes (2, 4, 8).

The second, more general, conclusion is that SA genes may be common in natural gene pools. The fact that SA genes accumulated in a small portion of the genome over the course of a microevolutionary study suggests that these genes may be present at low frequency at many loci that are widely dispersed throughout the genome. This would lead to a considerable conflict between the sexes being manifest at the level of the genome and adaptation by each sex would be compromised (sexual dimorphism load), owing to sex-specific selection and the fact that the sexes must share a common gene pool.

## REFERENCES AND NOTES


1. D. Charlesworth and B. Charlesworth, *Genet. Res.* **35**, 205 (1980).
2. J. J. Bull, *Evolution of Sex Determination Mechanisms* (Benjamin-Cummings, Menlo Park, CA, 1983); W. R. Rice, *Evolution* **38**, 735 (1984).
3. S. P. H. Mandel, *Heredity* **26**, 49 (1971); J. F. Kidwell *et al.*, *Genetics* **85**, 171 (1973).
4. R. A. Fisher, *Biol. Rev.* **6**, 345 (1931).
5. B. Charlesworth, J. A. Coyne, N. H. Barton, *Am. Nat.* **1987**, 113 (1987).
6. D. L. Lindsley and E. H. Grell, *Genetic Variations of Drosophila melanogaster* (Carnegie Institution of Washington, Washington, DC, 1968).
7. See for review, H. T. Speith and J. M. Ringo, in *Genetics and Biology of Drosophila*, M. Ashburner, H. L. Carson, J. N. Thompson, Jr., Eds. (Academic Press, San Francisco, 1983), vol. 3c, chap. 23.
8. B. Charlesworth, *Science* **251**, 1030 (1991).
9. To avoid overcrowding the males and females were separated before mating into five equal groups (pairs of 20 females and 30 males) placed into five half-pint plastic containers containing approximately 75 ml of standard molasses-cornmeal medium. Flies were allowed to lay eggs for 24 hours before being removed, and then, starting 9 days later, offspring were collected and pooled across the five bottles until sufficient numbers were obtained to begin the next generation.


10. Supported in part by NSF grant BSR-89-96268. I thank E. Hostert, D. Gessler, and J. Romo for technical assistance and A. Burt and R. Trivers for comments on the manuscript.

22 January 1992; accepted 3 April 1992


# Lyme Disease in California: A Novel Enzootic Transmission Cycle of *Borrelia burgdorferi*


Richard N. Brown and Robert S. Lane



**Knowledge of zoonotic transmission cycles is essential for the development of effective strategies for disease prevention. The enzootiology of Lyme disease in California differs fundamentally from that reported from the eastern United States. Woodrats, not mice, serve as reservoir hosts, and *Ixodes neotomae*, a nonhuman-biting tick, maintains the agent of Lyme disease, *Borrelia burgdorferi*, in enzootic cycles. The western black-legged tick, *Ixodes pacificus*, is the primary vector to humans, but it appears to be an inefficient maintenance vector. Isolates of *B. burgdorferi* from California exhibit considerable antigenic heterogeneity, and some isolates differ strikingly from isolates recovered from this and other geographic regions.**


Vector-borne zoonotic diseases are often maintained in complex transmission cycles involving several arthropod vectors and their wild vertebrate hosts. Comprehensive studies of the relative importance of potential reservoir hosts and their associated vectors provide insight into the basic mechanisms maintaining a zoonotic disease agent and may yield the knowledge necessary to avoid or control human disease.

Reservoirs of Lyme disease are defined as host species whose individuals are commonly infected, that perpetuate borrelial infections for prolonged periods, and that remain infective to vectors. Vector competence describes the inherent ability of an arthropod to become infected, to perpetuate, and to subsequently transmit the disease agent. The relative importance of competent reservoirs and vectors depends on the interaction of many variables and may differ between populations or communities of hosts and vectors.

The etiologic agent of Lyme disease, *Borrelia burgdorferi*, is maintained in enzootic cycles involving wildlife hosts and ticks throughout much of the temperate world. In the United States, ticks in the *Ixodes ricinus* "complex," including *Ixodes dammini* in the Northeast and Midwest and *Ixodes pacificus* in the West, have been shown to be the primary vectors to humans (1). *Ixodes dammini* is also the principle maintenance vector within cycles involving wild reservoirs, primarily the white-footed mouse, *Peromyscus leucopus*, in the East (1, 2), and it has been generally assumed that ticks in the *I. ricinus* "complex" are serving similar roles in other geographic areas. However, although *Ixodes pacificus* and *Peromyscus* spp. mice are common, they appear relatively unimportant in transmission cycles of *B. burgdorferi* in north coastal California. Rather, dusky-footed woodrats (*Neotoma fuscipes*, hereinafter referred to as woodrats) and a non–*I. ricinus* complex tick, *Ixodes neotomae*, support an enzootic cycle that maintains levels of *B. burgdorferi* in nature. The known geographic range of *I. neotomae* overlaps the distributions of *I. pacificus* and *N. fuscipes* in California; these species co-occur


Group in Parasitology and Department of Entomological Sciences, 218 Wellman Hall, University of California, Berkeley, CA 94720.

throughout western California, from Oregon to the extreme southern part of the state, and in the west-facing foothills of the Sierra Nevada Range (3, 4). This predominantly coastal mountain–Sierra Nevada foothills distribution also overlaps the area where most cases of Lyme disease occur in California.

A total of 102 of the first 105 borrelial isolates obtained from wild vertebrates in northern California (5) was cultured from biopsies (6) taken from either woodrats ($n$ = 72 of 165, 44%) or California kangaroo rats (*Dipodomys californicus*, hereinafter referred to as kangaroo rats, $n$ = 30 of 55, 55%) (7). Previously, serological surveys revealed evidence of high rates of exposure to borreliae in lagomorphs (8, 9), deer (10), and mice (11). However, no spirochetal isolates were obtained from tissue samples from 93 black-tailed deer (*Odocoileus hemionus columbianus*) or 48 black-tailed jackrabbits (*Lepus californicus*), and only three isolates were cultured from 165 (1.8%) *Peromyscus* spp. mice. Consistent isolation of spirochetes from woodrats and kangaroo rats, but rarely from *Peromyscus* spp. mice, contrasts sharply with reports from the eastern United States (12).

Woodrats remain infected, and infective to ticks, for over 1 year and are thus capable of maintaining infections between periods of vector abundance. We isolated spirochetes from woodrats and kangaroo rats that were trapped during all seasons, suggesting that these rodents either maintained their infections over time or that they were being reinfected periodically throughout the year. Five wild-caught, naturally infected woodrats retained their infections and remained infectious for ticks for 13 to 15 months (the duration of their captivity) (13, 14). Long-term infections suggest that infected woodrats may retain borreliae for the remainder of their lives. If so, infected woodrats may serve as a source of spirochetes for several cohorts of tick vectors.

Infection rates in field-collected ticks vary between species of potential vectors. Borreliae have been detected rarely in *Dermacentor occidentalis* (<1%) (1, 15, 16). *Ixodes pacificus* is a competent vector of *B. burgdorferi*, but infection rates in nature have been low (typically 1 to 2%; range, <1 to 5.9%) in both adult ticks flagged from vegetation (1, 10, 15, 17) and in immature ticks removed from hosts (16, 18). Infection rates in samples of *I. neotomae* removed from wild mammals were much higher than from any sample of *I. pacificus* reported (19); overall, 17 (15.2%) of 112 *I. neotomae* removed from three species of hosts were infected with spirochetes (Table 1). Significantly, all stages of this tick were infected at relatively high rates. Transstadial transmission (maintenance of an agent between successive life stages) was first demonstrated in *I. neotomae* when five of eight and three of four replete wild-caught larvae and nymphs, respectively, were determined to be infected after the transstadial molt. All isolates obtained from *I. neotomae* have been identified as *B. burgdorferi* (20).

**Table 1.** Borrelial infection rates in *Ixodes neotomae* removed from woodrats, kangaroo rats, and jackrabbits that were collected at the Hopland Field Station, Mendocino County, CA.

| Host species | Stage of *I. neotomae* | No. assayed | No. (%) positive |
|---|---|---|---|
| *Neotoma fuscipes* | Adults | 17 | 1 (5.9) |
| | Nymphs | 28 | 3 (10.7) |
| | Nymphs* | 4 | 3 (75.0) |
| | Larvae | 27 | 0 |
| | Larvae* | 7 | 5 (71.4) |
| *Dipodomys californicus* | Nymphs | 2 | 1 (50.0) |
| | Larvae | 6 | 0 |
| | Larva* | 1 | 0 |
| *Lepus californicus* | Adults† | 20 | 4 (20.0) |
| Total† | | 112 | 17 (15.2) |

*Collected as replete larvae and nymphs and tested approximately 6 weeks after the transstadial molt to nymphs and adults, respectively. All other immatures were assayed for infection as replete ticks prior to the transstadial molt. †Includes two of ten infected adults published previously (8).

The vector competence of *I. neotomae*, *I. pacificus*, and *D. occidentalis* was compared experimentally by feeding uninfected larvae of each species simultaneously on ten naturally infected woodrats and a kangaroo rat (21). After the transstadial molt, 23.8% of *I. pacificus* ($n$ = 80), 32.9% of *I. neotomae* ($n$ = 76), and 0% of *D. occidentalis* ($n$ = 44) were infected with *B. burgdorferi* (14). Although the proportions of infected *I. neotomae* and *I. pacificus* were not significantly different ($P$ = 0.1373, Fisher's exact test), both differed significantly from that of *D. occidentalis* ($P$ = 0.0001 and $P$ < 0.0001, respectively). Furthermore, individual *I. neotomae* nymphs were shown to transmit spirochetes efficiently to three different species of rodents (22). These experimental findings suggest that the vector competence of *I. neotomae* is as least as great as that of *I. pacificus*. However, the relative importance of *I. neotomae* and *I. pacificus* as vectors in enzootic transmission cycles of *B. burgdorferi* appears dramatically different because the natural histories of these two species differ in several important respects.

Little is known about the biology of *I. neotomae*; its host range appears to be limited to rodents and lagomorphs (3), and this tick is not known to feed on humans. We removed larval and nymphal *I. neotomae* from several species of rodents including woodrats, kangaroo rats, deer mice, and piñon mice, and adults from woodrats (23) and jackrabbits (5, 8, 23). Although all stages of *I. neotomae* feed on woodrats, the intensity of infestation (that is, the number of ticks per infested animal) is relatively low. Even during winter, the season when all stages are most abundant, the intensities of larval, nymphal, and adult *I. neotomae* removed from woodrats were only 2.0 (±1.6), 1.7 (±1.1), and 1.4 (±0.6), respectively (23, 24). However, low infestation rates can support enzootic cycles when infection rates in vectors are relatively high, and the level of infection in our sample of *I. neotomae* was 15.2%. Although this level of infection is lower than that commonly reported for samples of *I. dammini* in the eastern United States, it is much higher than reported previously for *I. pacificus* (19). Thus, *I. neotomae* appears to be able to maintain the cycle of *B. burgdorferi* enzootically.

At least two stages (larvae, nymphs, or adults) of a competent tick vector must feed commonly on the same reservoir host species to perpetuate a transmission cycle and maintain *B. burgdorferi* in nature. Larval *I. pacificus* are true generalists and feed on a variety of lizards, birds, and mammals (3). Nymphs feed on many species of small, medium, and large mammals (3), but we have rarely found them on *Peromyscus* spp. mice or other rodents (11, 16, 18). We removed only a single *I. pacificus* nymph from 294 woodrats (23). Conversely, western fence lizards, *Sceloporus occidentalis*, are parasitized often by larval and nymphal *I. pacificus* (18), but these lizards appear to be incompetent hosts of *B. burgdorferi* (25). Adults of *I. pacificus* normally feed on medium and large mammals, including jackrabbits and deer, and rarely feed on rodents (3). Transovarial transmission (transmission of spirochetes from an infected female through eggs to her progeny) can occur in *I. pacificus*, and would provide a mechanism by which maintenance could occur, but this route of transmission is rare (26).

The importance of *I. pacificus* as the primary maintenance vector of *B. burgdorferi* depends in part on the proportion of nymphs that feed on reservoir-competent hosts. In north coastal California, this proportion is too small for *I. pacificus* to serve as a primary maintenance vector in enzootic transmission cycles. However, transmission cycles may depend entirely on *I. pacificus* where lizards are absent or rare and, consequently, larvae and nymphs feed predominantly on reservoir-competent hosts. Cycles involving other species of ticks, such as *I. neotomae*, *Ixodes* *angustus*, and *Ixodes spinipalpis*, may be important in habitats where these ticks are relatively common (*27*). Different enzootic cycles may maintain *B. burgdorferi* in habitats that support different assemblages of potential vectors and reservoir hosts. Interactions between enzootic cycles may be quite complex, and the system maintaining *B. burgdorferi* should be envisioned as a web of transmission with multiple bridges between cycles and not simply as independent, parallel cycles.

In addition to differences in reservoir hosts and vectors, protein profiles of isolates of *B. burgdorferi* from California are more variable than, and often differ markedly from, those from the eastern United States (*28–30*). The range in variation in protein profiles (*20*) of California isolates of *B. burgdorferi* is illustrated in Fig. 1. Protein profiles of isolates obtained from mammals trapped within meters of each other, or from individual ticks removed from the same mammal, were often quite different (*23*). Isolates from California (as well as other regions) have been shown to vary in the presence and abundance of lower molecular weight proteins (≈20 to 26 kD) (*29, 30*). However, the protein profiles of several recent isolates (including CA 87, 139, and 146, Fig. 1) from wild-caught mammals or ticks lack major bands representing outer surface proteins (Osp) A and B and are so distinct from earlier isolates of *B. burgdorferi* that we were originally unsure of their specific identities.

Variation in the structure and abundance of surface proteins is biologically significant and should be considered in any evaluation of the efficacy of potential vaccines or serodiagnostic techniques and in studies of pathogenicity and the development of disease. Several authors have commented that European isolates of *B. burgdorferi* are typically more heterogeneous than isolates from the eastern United States (*28*). Isolates from California appear to be as antigenically heterogeneous as European isolates and more so than isolates from the eastern United States. Variant isolates increase our understanding of the range of variation in *B. burgdorferi*, but the significance of this variation, in terms of induced host immune response, pathogenicity, and the production of overt disease, is not yet understood.

In north coastal California, *B. burgdorferi* is maintained in an enzootic cycle involving *I. neotomae*, woodrats, and, in certain habitats, kangaroo rats. The narrow host range of *I. neotomae*, which apparently focuses on reservoir hosts, enhances the importance of this cycle. Because transovarial transmission of *B. burgdorferi* by *I. neotomae* has not been demonstrated, this cycle probably depends on horizontal transmission (that is, from tick to reservoir host to tick). Notably, all stages of *I. neotomae* feed primarily from late fall through early spring. Most transmission to reservoir hosts probably occurs several months before *I. pacificus* larvae are most active (*3, 18, 23*), and larval *I. pacificus* that acquire spirochetes while feeding on rodents would be potentially infective as nymphs and adults. However, since most *I. pacificus* nymphs and adults do not feed on known reservoir hosts, the transmission cycle does not usually progress. Nevertheless, *I. pacificus* larvae feed on reservoir hosts and are subsequently infected with *B. burgdorferi* as nymphs and adults (hence the transmission to humans).

The significance of potential reservoir species other than woodrats remains problematic. California kangaroo rats may be locally important reservoirs, but they are both ecologically and geographically restricted in distribution (*31*). Although *Peromyscus* spp. mice from California may be reservoir-competent (*23*), they do not serve as primary reservoirs in north coastal California because the nymphs of neither *I. pacificus* nor *I. neotomae* feed commonly on such mice (*11, 16, 18, 23*). In New York State, eastern cottontail rabbits (*Sylvilagus floridanus*) are reservoir-competent for *B. burgdorferi* and support an enzootic cycle involving *Ixodes dentatus* (*32*). In California, cottontail rabbits, *Sylvilagus* spp., and black-tailed jackrabbits are hosts to adults of *I. pacificus* and *I. neotomae* (*3, 8*); thus, these lagomorphs are exposed to *B. burgdorferi* and may be involved tangentially in transmission cycles. Black-tailed deer are exposed to *B. burgdorferi*, but, although serologic data suggest high rates of exposure in jackrabbits and deer, all attempts to isolate spirochetes from these hosts in California have been unsuccessful (*12*). The geographic ranges of other species of potential reservoirs, including two species of woodrats, ten species of kangaroo rats, and five species of *Peromyscus* mice, also overlap areas of California where Lyme disease is endemic. It seems likely that some of these rodents contribute to the local maintenance of *B. burgdorferi* (*12, 33*). Moreover, the reservoir competence of other species of woodrats should be evaluated in areas where Lyme disease occurs but where the enzootic maintenance cycles are unknown (*12*).

The control of ticks residing in woodrat nests may be facilitated by the nesting behaviors of woodrats (*12*), which collect

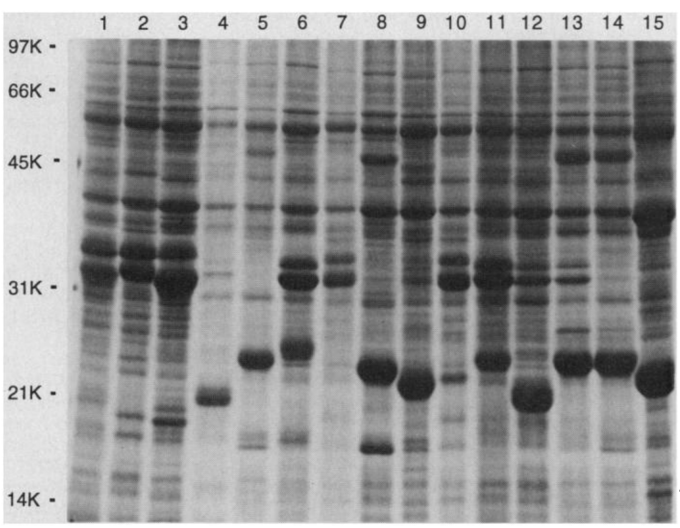


**Fig. 1.** Coomassie blue–stained SDS–polyacrylamide gel electrophoresis (PAGE) gel showing protein profiles of whole-cell lysates of the type strain of *Borrelia burgdorferi*, B31 (lane 1), the type strain of the relapsing fever spirochete, *Borrelia hermsii*, HS1 (lane 15), and isolates of *B. burgdorferi* isolated from ticks and mammals collected at the Hopland Field Station in Mendocino County, California (lanes 2 to 14). Lanes 2 and 3, CA 134 and 172 from *Ixodes pacificus*; lanes 4 to 6, CA 55, 139, and 140 from *Ixodes neotomae*; lanes 7 to 10, CA 32, 87, 122, and 123 from California kangaroo rats; lanes 11 to 14, CA 40, 102, 112, and 146 from dusky-footed woodrats. Most isolates of *B. burgdorferi*, including strain B31 and other isolates reported from the eastern United States, have major protein bands of ≈31 and ≈34 kD comprising Osp A and Osp B, respectively. Isolates CA 55, 122, 102, and 112 show banding patterns that are distinct from typical *B. burgdorferi*, and CA 87, 139, and 146 are representative of those isolates that lack major protein bands of ≈31 and ≈34 kD. These variant isolates resemble a clone (DN-127-C19-2) from an isolate cultured from an *I. pacificus* tick that was collected in Del Norte County, California (*30*). Isolates cultured from wild-caught rodents and ticks in California confirm that variant isolates are relatively common. Of 91 isolates compared by SDS-PAGE, 8 (9%) lacked both Osp A and B bands, 9 (10%) displayed the Osp A band (≈31 kD) but lacked the Osp B band, 31 (34%) had lower molecular weight protein bands (20 to 26 kD), and only 43 (47%) were similar to B31. All isolates of *B. burgdorferi* reacted with at least two of four monoclonal antibodies (H3TS, H5TS, H5332, and H6831) generated against *B. burgdorferi*, and none of them reacted with a monoclonal antibody specific to *B. hermsii* (H4825). Moreover, 14 of the most variant isolates (including CA 55, 87, 139, and 146) were confirmed by Western blot and DNA amplifications, by polymerase chain reaction, to be *B. burgdorferi* (*35*).

and store food items as well as other objects [possibly including nesting materials, such as cotton, impregnated with an acaricide, such as permethrin (*34*)] within their nests. Control of a relative specialist, such as *I. neotomae*, should be easier than the control of generalists, such as *I. dammini* or *I. pacificus*, which feed on a variety of hosts in diverse habitats. Direct reduction of woodrat populations would not control Lyme disease because *I. neotomae* has a broad enough host range to allow for shifts to alternative hosts. The control of enzootic cycles, by controlling specialist ticks locally, should be considered as a strategy to reduce the risk of humans acquiring Lyme disease in areas of California where *I. pacificus* is mainly an incidental maintenance vector of *B. burgdorferi*.

## REFERENCES AND NOTES


1. R. S. Lane, J. Piesman, W. Burgdorfer, *Annu. Rev. Entomol.* **36**, 587 (1991).
2. J. F. Levine, M. L. Wilson, A. Spielman, *Am. J. Trop. Med. Hyg.* **34**, 355 (1985); J. G. Donahue, J. Piesman, A. Spielman, *ibid.* **36**, 92 (1987); T. N. Mather, M. L. Wilson, S. I. Moore, J. M. Ribeiro, A. Spielman, *Am. J. Epidemiol.* **130**, 143 (1989).
3. D. P. Furman and E. C. Loomis, *Bull. Calif. Insect Surv.* **25**, 1 (1984).
4. L. G. Ingles, *Mammals of the Pacific States* (Stanford Univ. Press, Stanford, CA, 1965).
5. Most field investigations, including all collections of *I. neotomae*, were conducted at the University of California Hopland Field Station in southeastern Mendocino County. In addition, three isolates of *B. burgdorferi* were obtained from woodrats live-trapped near Ukiah, CA, and another was obtained from whole blood taken from a woodrat trapped near the Los Angeles basin (*33*). The primary study area is located centrally in the region of California where most cases of human Lyme disease originate; approximately 72% of Lyme disease cases identified in this state from 1983 through 1987 were thought to have been contracted in the four north coastal counties of Humboldt, Mendocino, Sonoma, and Marin [Infectious Disease Branch, Department of Health Services, *Calif. Morbid.* **47–48**, 1 (1991)].
6. Two 2-mm cylinders (one from the base of each ear) were punch-biopsied from rodents following R. J. Sinsky and J. Piesman [*J. Clin. Microbiol.* **27**, 1723 (1989)]. Rodents were anesthetized with methoxyflurane and handled in accordance with animal care and use protocols. Ear-punch tissue plugs were inoculated into 1.5-ml microcentrifuge tubes containing 1 ml of modified BSK II medium and 50 μg of rifampacin. Each culture was examined weekly for at least 5 weeks. Positive cultures were passed into fresh medium until high-density cultures were obtained.
7. The numbers of rodents that yielded spirochete-positive cultures included 7 of 18 (39%) kangaroo rats and 30 of 81 (37%) woodrats sampled during spring 1990, 3 of 3 (100%) kangaroo rats and 8 of 9 (89%) woodrats trapped during September 1990, 1 of 2 (50%) kangaroo rats and 10 of 22 (45%) woodrats sampled during December 1990, and 4 of 4 (100%) kangaroo rats and 11 of 24 (46%) woodrats trapped during February 1991, no ($n$ = 1) kangaroo rats and 1 of 6 (17%) woodrats sampled during August 1991, 5 of 11 (45%) kangaroo rats, 7 of 14 (50%) woodrats, 1 of 4 (25%) piñon mice, and 1 of 6 (17%) brush mice trapped during late November 1991, 10 of 16 (62%) kangaroo rats, 5 of 9 (55%) woodrats, and 1 of 10 (10%) piñon mice sampled during February 1992 (these data include 14 recaptures with 4 reisolations from kangaroo rats and 55 recaptures with 15 reisolations from woodrats).
8. R. S. Lane and W. Burgdorfer, *J. Wildl. Dis.* **24**, 1 (1988).
9. R. S. Lane and D. C. Regnery, *ibid.* **25**, 189 (1989).
10. R. S. Lane and W. Burgdorfer, *Zentralbl. Bakteriol. Mikrobiol. Hyg.* **A 263**, 55 (1986).
11. R. S. Lane, *Bull. Soc. Vector Ecol.* **15**, 25 (1990).
12. _____ and R. N. Brown, *J. Med. Entomol.* **28**, 299 (1991).
13. To determine whether woodrats retain borrelial infections for prolonged periods, five pregnant, naturally infected woodrats were housed individually at the University of California, Berkeley, animal facilities where they were allowed to birth and rear their young (in accordance with animal care and use protocols). These woodrats were not reexposed to *B. burgdorferi* while in captivity. Infections were monitored by periodically (not more often than once per month) culturing ear-punch biopsies from each animal (*6*). Additionally, each woodrat was infested with 100 clean-colony *I. pacificus* larvae after an average of 8 and 14 months following capture. All five woodrats retained their infections throughout captivity and infected clean-colony larval ticks during both infestations; borrelial infections were detected in 11.1% and 21.7% of the resultant nymphs from the two respective feedings.
14. Larvae and most nymphal ticks were smeared in toto onto glass microscope slides and examined by direct immunofluorescence (DI) (*18*). Some nymphs (including those assayed during the experiment comparing vector competence) and all adults were embedded individually in paraffin and dissected. A portion of midgut (or half of the bodily tissues of nymphs) from each dissected tick was examined by DI and another portion of midgut (or the other half of the tissues) was inoculated into BSK II medium. Cultures were examined weekly by dark-field microscopy for at least 5 weeks.
15. R. S. Lane and P. E. Lavoie, *Ann. N.Y. Acad. Sci.* **539**, 192 (1988).
16. R. S. Lane and J. E. Loye, *J. Med. Entomol.* **28**, 719 (1991).
17. W. Burgdorfer, R. S. Lane, A. G. Barbour, R. A. Gresbrink, J. R. Anderson, *Am. J. Trop. Med. Hyg.* **34**, 925 (1985).
18. R. S. Lane and J. E. Loye, *J. Med. Entomol.* **26**, 272 (1989).
19. Direct comparison of infections in ticks flagged from vegetation with infections in ticks removed from hosts, or even between ticks removed from different species of hosts (due to differences in reservoir competence), is difficult because ticks become infected when feeding on infected hosts. Unfortunately, we have been unable to collect unfed *I. neotomae* for comparison with *I. pacificus* flagged from vegetation.
20. Isolates were identified by their reactivities to six monoclonal antibodies (H9724, reactive with all *Borrelia* species; H3TS, H5TS, H5332, and H6831 generated against *B. burgdorferi*; and H4825 specific to *B. hermsii*) using an indirect immunofluorescence assay (IFA) and by SDS-PAGE (*29*). Variant isolates were analyzed by several different genetic analyses including amplification of DNA by the polymerase chain reaction (*35*). Isolates were characterized after a few in vitro passages (mean, 4.7; range, 3 to 8) to minimize potential changes in antigenic and genetic characteristics.
21. Approximately 100 clean-colony larval *I. pacificus*, *I. neotomae*, and *D. occidentalis* were simultaneously put on each of ten naturally infected woodrats and a naturally infected kangaroo rat. Infested animals were housed in cages suspended above water and replete larvae were collected daily. Ticks were maintained under constant conditions (21°C and ≈98% relative humidity) and dissected approximately 6 weeks following the molt to the nymphal stage. Numbers of ticks differed between species because of differences in the numbers of each species that fed and survived. The numbers of *I. pacificus* and *I. neotomae* were paired by host and day of drop-off as nearly as possible. Most of the replete *D. occidentalis* larvae died, and all survivors ($n$ = 44) were tested.
22. Rates of infection were estimated for different batches of *I. neotomae* nymphs fed on infected woodrats as larvae. Potentially infected nymphs were fed on previously unexposed hosts including two Syrian hamsters, two piñon mice, one deer mouse, and four woodrats. Replete nymphs were collected daily and tested for infections (*14*) approximately 2 weeks after drop-off to determine the exact number of infected *I. neotomae* nymphs that had fed on each host. Four animals (two hamsters, one piñon mouse, and one woodrat) were fed on by one infected *I. neotomae* nymph, and two infected nymphs fed on three woodrats. Spirochetes were later isolated from all seven animals fed on by infected *I. neotomae* nymphs.
23. R. N. Brown and R. S. Lane, unpublished data.
24. The larval and nymphal stages of *I. neotomae*, which have not been described previously, were identified by comparison with voucher specimens from a laboratory colony. The numbers of ticks on wild woodrats were estimated by visual counts and removal of feeding ticks.
25. R. S. Lane, *Am. J. Trop. Med. Hyg.* **42**, 75 (1990).
26. _____ and W. Burgdorfer, *ibid.* **37**, 188 (1987); G. B. Schoeler and R. S. Lane, unpublished data.
27. T. Damrow, H. Freedman, R. S. Lane, and K. L. Preston [*West. J. Med.* **150**, 580 (1989)] implicated *I. angustus* as a vector of *B. burgdorferi* in Washington State. *Ixodes spinipalpis* is similar morphologically to *I. neotomae* and ecologically to both *I. dentatus* (*32*) and *I. neotomae* in that all three species feed heavily on lagomorphs. *Ixodes angustus*, *I. neotomae*, and *I. spinipalpis* occur in California, but this is the first time vector competence has been reported for any of these species.
28. A. G. Barbour, S. L. Tessier, S. F. Hayes, *Infect. Immunol.* **45**, 94 (1984); A. G. Barbour, R. A. Heiland, T. R. Howe, *J. Infect. Dis.* **152**, 478 (1985); J. F. Anderson *et al.*, *J. Clin. Microbiol.* **27**, 13 (1989); R. B. LeFebvre, R. S. Lane, G.-C. Perng, J. A. Brown, R. C. Johnson, *ibid.* **28**, 700 (1990); J. F. Anderson, *Scand. J. Infect. Dis.* **77** (suppl.), 23 (1991); B. Wilske *et al.*, *ibid.*, p. 108.
29. R. S. Lane and J. A. Pascocello, *J. Clin. Microbiol.* **27**, 2344 (1989).
30. M. L. Bissett and W. Hill, *ibid.* **25**, 2296 (1987); M. Bissett, W. Hill, W. Probert, S. Kurashige, *Ann. N.Y. Acad. Sci.* **539**, 369 (1988); S. Kurashige, M. Bissett, L. Oshiro, *J. Clin. Microbiol.* **28**, 1362 (1990).
31. D. A. Kelt, *Mamm. Species* **324**, 1 (1988).
32. S. R. Telford III and A. Spielman, *Am. J. Trop. Med. Hyg.* **41**, 482 (1989).
33. W. M. Boyce, R. N. Brown, B. C. Zingg, R. B. LeFebvre, R. S. Lane, *J. Med. Entomol.* **29** (no. 3), 496 (1992).
34. T. N. Mather, J. M. C. Ribeiro, A. Spielman, *Am. J. Trop. Med. Hyg.* **36**, 609 (1987).
35. T. G. Schwan, R. N. Brown, R. S. Lane, unpublished data. Following Western blotting to nitrocellulose paper, variant isolates ($n$ = 14 tested), including CA55, 87, 139, and 146 shown in Fig. 1, reacted with monoclonal antibodies (H5332 and H3TS) specific for *B. burgdorferi*. DNA sequences of all variant isolates were amplified by the polymerase chain reaction using nested primers specific for *B. burgdorferi* [P. A. Rosa, D. Hogan, T. G. Schwan, *J. Clin. Microbiol.* **29**, 524 (1991)].


36. We thank J. A. Pascocello and J. Kleinjan for technical support, S. Glickman and D. Berger for advice and assistance in colonizing woodrats, G. B. Schoeler and T. G. Schwan for permission to cite unpublished data, A. G. Barbour and T. G. Schwan for generously supplying monoclonal antibodies, and J. O. Washburn, W. M. Boyce, and G. B. Schoeler for reviewing earlier drafts of this manuscript. Funding was provided in part by two University of California, School of Public Health, Doctoral Student Support Awards to R.N.B., U.S. Public Health Service grant AI22501 to R.S.L., Centers for Disease Control grant U50/CCU906594 to R.S.L., and the Margaret C. Walker Foundation.

10 January 1992; accepted 11 April 1992