Population Genetic Structure of *Ixodes pacificus* (Acari: Ixodidae) Using Allozymes

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ABSTRACT Genetic analysis of the population structure of the western blacklegged tick, *Ixodes pacificus* Cooley & Kohls, was conducted using allozymes. This vector tick transmits the Lyme disease spirochete, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner, in the far-western United States. It ranges from British Columbia to Baja California and disjunct populations are present in Oregon, Nevada, Utah, and Arizona. Host-seeking adult ticks were collected from vegetation across the range of the species and were partially fed on rabbits prior to analysis. Twelve putative loci were resolved using starch gel electrophoresis. One locus, glucose-6-phosphate isomerase, formed an apparent north/south latitudinal cline and showed significant geographic structure. None of the remaining loci exhibited much genetic differentiation. Estimates of gene flow were high relative to other arthropods. Isolation-by-distance analysis suggests a recent and rapid range expansion. We conclude that the overall lack of differentiation is due high rates of gene flow.

KEY WORDS *Ixodes pacificus*, allozymes, population genetics, gene flow

THERE IS GROWING need to incorporate knowledge of population structure into epidemiological studies of arthropod-borne diseases (Tabachnick and Black 1995). Such investigations can help elucidate how evolutionary processes in both vectors and pathogens interact (Hilburn and Sattler 1986b, Oliver et al. 1993, Tabachnick and Black 1995). The western blacklegged tick, *Ixodes pacificus* Cooley & Kohls, is the primary vector of the Lyme disease spirochete, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner, in western North America (Burgdorfer et al. 1985) and may transmit the rickettsial pathogen *Ehrlichia equi* as well (Richter et al. 1996). The host preferences and nuclear DNA sequences of this tick vary geographically (Arthur and Snow 1968, Arnason 1992, Wesson et al. 1993, McLain et al. 1995), although the species has not been treated taxonomically for 50 yr (Cooley and Kohls 1945). A taxonomic controversy surrounding *Ixodes scapularis* Say, the eastern North American vector of *B. burgdorferi*, recently has had an impact on understanding the epidemiology of Lyme disease in this region (Oliver et al. 1993). Here we assess population structure, using allozymes, of *I. pacificus*.

Ixodes pacificus is distributed broadly from British Columbia south through Washington, Oregon, California, and into Baja California Norte, Mexico (Fig. 1). It occurs on Vancouver Island, British Columbia, and on several of the Channel Islands in California. Eastward, disjunct populations occur in Nevada, Utah, Arizona, and eastern Oregon (Bishopp and Trembley 1945, Gregson 1956, Allred et al. 1960, Furman and Loomis 1984, Olson et al. 1992).

The current distribution of *I. pacificus* may be the result of complex historical and contemporary factors. The disjunct populations in the Great Basin and Range area suggest the type of Pleistocene refugia observed for many other organisms (Hewitt 1993, Britten et al. 1995, Green et al. 1996). Alternatively, the distributional patterns of this tick may have resulted from recent range expansion. Because *I. pacficus* has a broad host range, which includes 80 species of reptiles, birds, and mammals, the potential for gene flow is high (Lane et al. 1991).

The purpose of this study was to use allozymes to survey the extent and pattern of population variation in *I. pacificus*.

Materials and Methods

Tick Collection and Preparation. Collecting localities are shown in Table 1 and Fig. 1. We were not able to collect *I. pacificus* in Nevada. Host-seeking adult ticks were collected from vegetation with flannel tick-drags and returned alive to the laboratory for processing.

Only female *I. pacificus* were used in the allozyme analysis because males were too small to obtain enough homogenate for electrophoresis. To induce the aconitase enzyme system, females were partially fed for 3–6 d (mean 4 d) on New Zealand

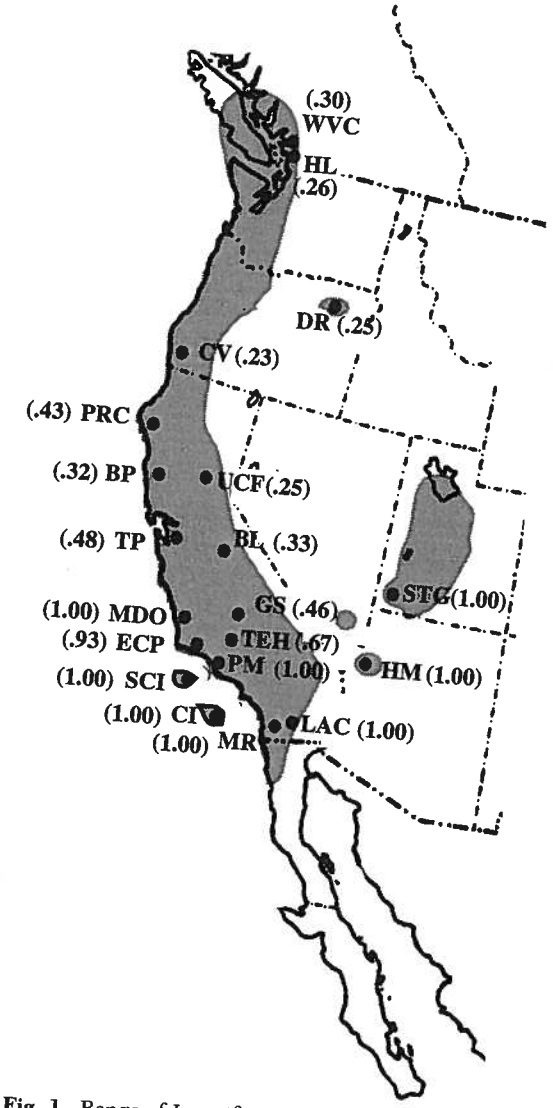

Fig. 1. Range of *I. pacificus* in western North America showing frequencies of GPI allele (A). Collecting localities are indicated with black circles.

white rabbits. Incompletely fed ticks were removed manually with forceps, placed in cryotubes, quick-frozen on dry ice, and stored at −70°C. *Ixodes spinipalpis* Hadwen & Nuttall, a close relative of *I. pacificus*, was used as an outgroup comparison (Klompen et al. 1996).

Allozyme Electrophoresis. Ticks were partially thawed and processed using 1 of 2 methods. The abdomens of highly engorged ticks were sliced open with sterile scalpels and their bloodmeals extruded. Tick bodies were then placed into wells of cold spotting plates. Ticks that were engorged only slightly or moderately were put directly into spotting plate wells without lancing. Twelve microliters of cold homogenization buffer (0.01 M Tris base, 0.001 M EDTA, pH 7.0) was added to each sample well and ticks were homogenized with cold ground-glass pestles. Homogenates were absorbed onto either 3 by 6 or 3 by 9 mm wicks (Whatman No. 1 filter paper) and loaded into the cathodal ends of 12.5% starch gels either 6 or 9 mm thick (potato starch, Sigma, St. Louis, MO, catalog No. S-5651).

The following buffer systems were used (Hilburn and Sattler 1986a): NAM, pH 6.5 and NAM, pH 7.1 [gel buffers, 0.002 M citrate; electrode buffers, 0.04 M citrate, both buffers corrected to pH with N-(3-amino-propyl)-morpholine], Tris-citrate, pH 8.1 [gel buffer, 0.017 M Tris base, 0.002 M citrate, pH 8.1; electrode buffer, 0.229 M Tris base, 0.052 M citrate, pH 8.1]. Starch gels were electrophoresed at 4°C for 2.5–4 h at 35 mA. After electrophoresis, gels were cut into 2-mm slices (3–5 slices per gel depending on thickness). Each slice was stained for a specific enzyme, then scored for the presence of electromorphs (alleles) relative to a known standard sample. Rabbit-host blood also was electrophoresed and analyzed to control for comigrating contaminating electromorphs. Any loci that comigrated with rabbit electromorphs were eliminated from the analyses.

Twelve putative loci were evaluated. The enzymes examined and the buffers used to resolve them are NAM, pH 6.5: aconitase-1 and −2 (ACOH-1, ACOH-2, EC 4.2.1.3); glucose-6-phosphate isomerase (GPI, 5.3.1.9), glycerol-3-phosphate dehydrogenase (G3PDH, 1.1.1.8); malate dehydrogenase-1 and −2 (MDH-1, MDH-2, 1.1.1.37). NAM, pH 7.1: $NADP^+$ dependent malate dehydrogenase (MDHP, 1.1.1.40); fumarate hydratase (FUMH, 4.2.1.2). Tris-citrate, pH 8.1: adenylate kinase (AK, 2.7.4.3); fructokinase (FK); isocitrate dehydrogenase-1 and −2 (IDH-1, IDH-2, 1.1.1.42). Initially, 8 other enzymatic systems also were examined: esterase, glutathione reductase, glucose-6-phosphate dehydrogenase, guanine deaminase, lactate dehydrogenase, mannose-6-phosphate isomerase, peptidase, and superoxide dismutase. These systems were not employed, however, because they could not be scored reliably or because they comigrated with rabbit electromorphs.

Analyses. Preliminary results revealed that there was noticeable genetic differentiation between a group of northern populations (WVC, HL, DR, CV, PRC, UCF, BP, TP, BL, GS, TEH) and a group of southern populations (MDO, ECP, PM, SCI, CI, MR, LAC, HM, STG). Therefore, calculations of *F* statistics, estimates of gene flow and examination of relationships among populations were completed both by combining all tick populations and by separating the populations into the northern group and the southern group.

Allelic frequencies and variability measures were computed with BIOSYS 1.7 (Swofford and Selander 1981). Exact tests in GENEPOP were used to check for deviations from Hardy–Weinberg equilibrium, to test for linkage disequilibrium, and to

Table 1. Collecting localities and l

Code	State/ Province	County	
WVC	BC	NA	West
HL	BC	NA	Uppe
DR	OR	Wasco	High
CV	OR	Douglas	High
PRC	CA	Mendocino	High
UCF	CA	Yuba	UC S Ce
BP	CA	Mendocino	UC F
TP	CA	Alameda	Berke
BL	CA	Madera	Bass
GS	CA	Kern	High Cr
MDO	CA	San Luis Obispo	Mont km
TEH	CA	Kern	Teha alo
ECP	CA	Santa Barbara	El C
PM	CA	Venutra	Pt. M
SCI	CA	Santa Barbara	Santa
CI	CA	Los Angeles	Santa
MR	CA	San Diego	High
LAC	CA	San Diego	Cuya Ca
HM	AZ	Mohave	Hual
STG	UT	Washington	Dixie Gr

All collections were made by D. Kain ex

inspect for genic and genotyp (Raymond and Rousset 1995). method was used to estimate, w value associated with each exa Thompson 1992). To assess gen an exact test comparing all pairs performed using GENEPOP.

F statistics were calculated program (Goudet 1995). The 3 *F* fractional reduction in heterozyg cal inbreeding (f), genetic drift bination of inbreeding and gene and Cockerham 1984). The FS mates the means and variances using the jackknifing procedure the null hypothesis that $F = \theta$ tation procedure was performed Bonferroni test was added to adj type-I error rate (Rice 1989).

Gene flow estimates were methods. Using GENEPOP (Ra set 1995), the private alleles met Barton and Slatkin 1986) was e ond method of estimating ge equation: $Nm = (1-F_{st})/4\ F_{st}$ (w mator of F_{st}; Weir 1996). In add of isolation by distance was asse method (1993). In the Slatkin pairwise Nm values were regr pairwise distances. FSTAT was pairwise Nm values. Distances lometers) were taken from a n

Table 1. Collecting localities and locality codes for all *I. pacificus* examined

Code	State/ Province	County	Locality	Collection date	*n*
WVC	BC	NA	West Vancouver, Eagleridge Viewpoint	6, 8 Apr. 1992	20
HL	BC	NA	Upper Fraser Valley, Harrison Lake	6 Apr. 1993	17
DR	OR	Wasco	Highway 216, Sherar Bridge, Deschutes River Road	1 Apr. 1992; 1 Mar. 1995	4
CV	OR	Douglas	Highway 5, 5 km S Canyonville, Turkey Creek Road	31 Mar. 1992; 3 Mar. 1995	15
PRC	CA	Mendocino	Highway 271, 1.2 km N Highway 101	26 Mar. 1992	8
UCF	CA	Yuba	UC Sierra Foothill Range Research and Extension Center	27 Feb. 1995	10
BP	CA	Mendocino	UC Hopland Research and Extension Center	Mar. 1995	19
TP	CA	Alameda	Berkeley, Tilden Regional Park	Feb. 1994; Mar. 1995	65
BL	CA	Madera	Bass Lake, Road 222, N North Fork	14, 16 Feb. 1995	15
GS	CA	Kern	Highway 155, W Green Horn Summit, road to Alder Creek Campground	11 Mar. 1992; 12 Feb. 1995	12
MDO	CA	San Luis Obispo	Montana de Oro State Park, Pecho Valley Road, 3.8 km S park entrance	3 Feb. 1993	5
TEH	CA	Kern	Tehachapi, near Tehachapi Mountain County Park, along Water Canyon Road	12 Feb. 1995	6
ECP	CA	Santa Barbara	El Capitan State Park campground	4 Feb. 1993	7
PM	CA	Venutra	Pt. Magu State Park, Big Sycamore Canyon Trail	8–10 Mar. 1992; 9 Feb. 1993	14
SCI	CA	Santa Barbara	Santa Cruz Island, road to Prisoner's Harbour	6 Mar. 1992; 5, 6 Feb. 1993	10
OI	CA	Los Angeles	Santa Catalina Island, Avalon Canyon	13 Feb. 1993	15
MR	CA	San Diego	Highway 15, Mercy Road exit, along bike paths	4 Mar. 1992; 16 Feb. 1993; 10 Feb. 1995	20
LAC	CA	San Diego	Cuyamaca Ranch State Park, La Cima Conservation Camp	18 Feb. 1993; 11 Feb. 1995	20
HM	AZ	Mohave	Hualapai Mountain State Park, Boy Scout Camp	5 May 1992	7
STG	UT	Washington	Dixie National Forest, N of Silver Reef, road to Oak Grove Campground	22 Dec. 1996; 11 Apr. 1996	4

All collections were made by D. Kain except for those by D. Kindree (HL) and K. Padgett (BP). NA, not applicable. *n*, sample size.

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:. Homogenates were absorbed
or 3 by 9 mm wicks (Whatman
and loaded into the cathodal
ch gels either 6 or 9 mm thick
na, St. Louis, MO, catalog No.

uffer systems were used (Hil-
986a): NAM, pH 6.5 and NAM,
:, 0.002 M citrate; electrode buf-
e, both buffers corrected to pH
ropyl)-morpholine], Tris-citrate,
r, 0.017 M Tris base, 0.002 M
electrode buffer, 0.229 M Tris
rate, pH 8.1]. Starch gels were
t 4°C for 2.5–4 h at 35 mA. After
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specific enzyme, then scored for
electromorphs (alleles) relative to
d sample. Rabbit-host blood also
sed and analyzed to control for
ntaminating electromorphs. Any
rated with rabbit electromorphs
from the analyses.
ve loci were evaluated. The en-
l and the buffers used to resolve
M, pH 6.5: aconitase-1 and −2
DH-2, EC 4.2.1.3); glucose-6-phos-
e (GPI, 5.3.1.9), glycerol-3-phos-
genase (G3PDH, 1.1.1.8); malate
-1 and −2 (MDH-1, MDH-2,
I, pH 7.1: $NADP^+$ dependent mal-
nase (MDHP, 1.1.1.40); fumarate
MH, 4.2.1.2). Tris-citrate, pH 8.1:
se (AK, 2.7.4.3); fructokinase (FK);
ydrogenase-1 and −2 (IDH-1,
2). Initially, 8 other enzymatic sys-
examined: esterase, glutathione re-
se-6-phosphate dehydrogenase, gua-
se, lactate dehydrogenase, mannose-
somerase, peptidase, and superoxide
hese systems were not employed,
use they could not be scored reliably
hey comigrated with rabbit electro-

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Exact tests in GENEPOP were used to
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test for linkage disequilibrium, and to

inspect for genic and genotypic differentiation (Raymond and Rousset 1995). A Markov chain method was used to estimate, without bias, the *P* value associated with each exact test (Guo and Thompson 1992). To assess genic differentiation, an exact test comparing all pairs of populations was performed using GENEPOP.

F statistics were calculated using the FSTAT program (Goudet 1995). The 3 *F* statistics measure fractional reduction in heterozygosity caused by local inbreeding (f), genetic drift (θ), or the combination of inbreeding and genetic drift (F) (Weir and Cockerham 1984). The FSTAT package estimates the means and variances for these statistics using the jackknifing procedure over loci. To test the null hypothesis that $F = \theta = f = 0$, a permutation procedure was performed and a sequential Bonferroni test was added to adjust for group-wide type-I error rate (Rice 1989).

Gene flow estimates were calculated by two methods. Using GENEPOP (Raymond and Rousset 1995), the private alleles method (Slatkin 1985, Barton and Slatkin 1986) was employed. The second method of estimating gene flow used the equation: $Nm = (1\text{-}F_{st})/4\ F_{st}$ (where θ is the estimator of F_{st}; Weir 1996). In addition, the presence of isolation by distance was assessed using Slatkin's method (1993). In the Slatkin procedure, $\log_{10}$ pairwise Nm values were regressed against $\log_{10}$ pairwise distances. FSTAT was used to calculate pairwise Nm values. Distances (straight-line in kilometers) were taken from a map (Albers equal-area projection). Microsoft Excel (Microsoft 1994) was used to perform a simple linear regression.

Distance measures were calculated and phenograms were produced to assess genetic similarity relationships among component populations. Rogers' (1972, as modified by Wright 1978) and Nei's unbiased distance (1978) measures were calculated using BIOSYS. Distance measures were clustered by the unweighted pair group method using arithmetic averages.

Results

Polymorphism. Eight of the 12 loci examined were polymorphic within *I. pacificus*: ACOH-1, ACOH-2, FUMH, GPI, G3PDH, IDH-2, MDH-1, and MDH-2 (Table 2). The most polymorphic locus was ACOH-1 with 5 alleles, followed by ACOH-2 with 4 alleles, MDH-2 with 3 alleles, and each of the remaining polymorphic loci had 2 alleles. The percentage of polymorphic loci in each population ranged from 8.3 to 41.7 with a mean of 25.0. Average heterozygosity in each population ranged from a low of 0.046 to a high of 0.143, with a mean of 0.101. Unique alleles were found in the PRC and UCF populations. Allele B of FUMH occurred only in the PRC population and allele B of the MDH-1 locus was found only in the UCF population. There were 2 fixed allelic differences between *I. pacificus* and *I. spinipalpis*, at the G3PDH and MDHP loci (Table 2).

Table 2. Alleles and allelic frequencies for 8 loci that showed variation in or between *I. pacificus* (population codes as in Fig. 1) and *I. spinipalpis* (*I.* sp.)

Locus	Allele	Populations										
		WVC	HL	DR	CV	PRC	UCF	BP	TP	BL	GS	*I.* sp.
% polymorphism[a]		33.3	25.0	16.7	33.3	33.3	41.7	25.0	25.0	33.3	25.0	16.7
Avg heterozygosity		0.105	0.091	0.080	0.094	0.136	0.123	0.118	0.104	0.135	0.127	0.086
ACOH-1	A	0.80	0.65	0.38	0.36	—	0.50	0.60	0.69	0.47	0.26	—
ACOH-1	B	0.20	0.35	0.62	0.64	0.67	0.50	0.40	0.31	0.53	0.43	0.25
ACOH-1	C	—	—	—	—	0.33	—	—	—	—	—	—
ACOH-1	D	—	—	—	—	—	—	—	—	—	0.31	0.50
ACOH-1	E	—	—	—	—	—	—	—	—	—	—	0.25
ACOH-1	F	—	—	—	—	—	—	—	—	—	—	—
Sample size (*n*)		15	17	4	11	3	10	17	57	15	12	2
ACOH-2	A	0.80	0.91	1.00	0.92	0.81	0.80	0.76	0.87	0.63	0.83	0.25
ACOH-2	B	0.08	0.09	—	—	0.06	—	0.11	0.06	0.30	0.08	0.75
ACOH-2	C	0.12	—	—	0.08	0.13	0.15	0.13	0.07	0.07	—	—
ACOH-2	D	—	—	—	—	—	0.05	—	—	—	0.09	—
Sample size (*n*)		20	17	4	13	8	10	19	63	15	12	2
FUMH	A	1.00	1.00	1.00	1.00	0.88	1.00	1.00	1.00	1.00	1.00	1.00
FUMH	B	—	—	—	—	0.12	—	—	—	—	—	—
Sample size (*n*)		20	17	4	15	8	10	19	64	15	12	2
GPI	A	0.30	0.26	0.25	0.23	0.43	0.25	0.32	0.48	0.33	0.46	—
GPI	B	0.70	0.74	0.75	0.77	0.57	0.75	0.68	0.52	0.67	0.54	1.00
Sample size (*n*)		20	17	4	15	7	10	19	64	15	12	2
G3PDH	A	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	0.93	1.00	—
G3PDH	B	—	—	—	0.07	—	—	—	—	0.07	—	—
G3PDH	C	—	—	—	—	—	—	—	—	—	—	1.00
Sample size (*n*)		10	17	4	15	8	10	10	59	15	9	2
IDH-2	A	0.95	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00
IDH-2	B	0.05	—	—	—	—	0.05	—	—	—	—	—
Sample size (*n*)		20	17	4	15	8	10	19	55	15	12	2
MDH-1	A	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00
MDH-1	B	—	—	—	—	—	0.05	—	—	—	—	—
Sample size (*n*)		20	17	4	15	8	10	19	64	15	12	2
MDH-2	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MDH-2	B	—	—	—	—	—	—	—	—	—	—	—
Sample size (*n*)		20	17	4	15	8	10	19	64	15	12	2
MDHP	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	—
MDHP	B	—	—	—	—	—	—	—	—	—	—	1.00
Sample size (*n*)		20	17	4	14	8	10	19	49	15	12	2

Locus	Allele	MDO	TEH	ECP	PM	SCI	CI	MR	LAC	HM	STG
% polymorphism[a]		16.7	33.3	25.00	16.7	16.7	8.3	25.0	25.0	25.0	16.7
Avg heterozygosity		0.046	0.143	0.094	0.081	0.088	0.062	0.107	0.091	0.097	0.086
ACOH-1	A	0.80	0.42	0.60	0.62	0.45	0.44	0.47	0.44	0.07	0.75
ACOH-1	B	0.20	0.50	0.20	0.19	0.40	0.40	0.40	0.56	0.64	0.25
ACOH-1	C	—	—	—	—	0.05	0.08	—	—	0.29	—
ACOH-1	D	—	0.08	0.20	—	0.05	—	—	—	—	—
ACOH-1	E	—	—	—	0.19	0.05	0.08	0.13	—	—	—
Sample size (*n*)		5	6	5	14	10	14	13	16	7	4
ACOH-2	A	0.90	0.75	0.79	0.84	0.75	1.00	0.69	0.72	0.79	0.67
ACOH-2	B	—	0.17	—	0.16	0.25	—	0.22	0.20	—	0.25
ACOH-2	C	0.10	0.08	0.21	—	—	—	0.09	0.08	0.21	0.08
ACOH-2	D	—	—	—	—	—	—	—	—	—	—
Sample size (*n*)		5	6	7	14	10	15	16	20	7	4
FUMH	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
FUMH	B	—	—	—	—	—	—	—	—	—	—
Sample size (*n*)		5	6	7	14	10	15	18	20	7	4
GPI	A	1.00	0.67	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GPI	B	—	0.33	0.07	—	—	—	—	—	—	—
Sample size (*n*)		5	6	7	14	10	15	20	20	7	4
G3PDH	A	1.00	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00
G3PDH	B	—	—	—	—	—	—	0.05	—	—	—
Sample size (*n*)		5	6	7	14	10	15	20	19	7	4

Table 2. Continued

Locus	Allele	MDO	TE
IDH-2	A	1.00	1.0
IDH-2	B	—	—
Sample size (*n*)		5	
MDH-1	A	1.00	1.0
MDH-1	B	—	—
Sample size (*n*)		5	
MDH-2	A	1.00	0.
MDH-2	B	—	0.
Sample size (*n*)		5	

See text for locus abbreviations. [a]Allelic

Three loci, ACOH-1, ACOH- deviated from Hardy–Weinberg some populations, in all cases du deficiency ($P < 0.05$). ACOH-1 to Hardy–Weinberg equilibrium i CV, TP, CI, MR, LAC, and HM explained in Table 1). ACOH-2 v Weinberg equilibrium in the po GS, PM, SCI, and LAC. Finally, in 3 populations: CV, BL, and M equilibria were found among all

Glucose-6-Phosphate Isome differentiation occurred at the G a northern (GPI allele A ≤ 0.6 group of populations (GPI allel 1). Additionally, a rough north cline was found in GPI (Fig. 1) creases in frequency from nort becomes fixed in the MDO, P LAC, HM, and STG popul ACOH-1, ACOH-2 and GPI sho < 0.05) differentiation among However, ACOH-1 and ACOH- hibited no obvious geographic patterns of dissimilarity.

Table 3. *F* statistics for the popu

Locus
ACOH-1
ACOH-2
FUMH
GPI
G3PDH
IDH-2
MDH-1
MDH-2
All loci, all localities
Jackknifing, all localities
All loci, northern group
Jackknifing, northern group
All loci, southern group
Jackknifing, southern group

Results are given by locus, over all loci $P < 0.05$ that F, θ and $f = 0$.

between *I. pacificus* (population

TP	BL	GS	*I.* sp.
25.0	33.3	25.0	16.7
0.104	0.135	0.127	0.086
0.69	0.47	0.26	—
0.31	0.53	0.43	0.25
—	—	—	—
—	—	—	—
—	—	0.31	0.50
—	—	—	0.25
57	15	12	2
0.87	0.63	0.83	0.25
0.06	0.30	0.08	0.75
0.07	0.07	—	—
—	—	0.09	—
63	15	12	2
1.00	1.00	1.00	1.00
—	—	—	—
64	15	12	2
0.48	0.33	0.46	—
0.52	0.67	0.54	1.00
64	15	12	2
1.00	0.93	1.00	—
—	0.07	—	—
—	—	—	1.00
59	15	9	2
1.00	1.00	1.00	1.00
—	—	—	—
55	15	12	2
1.00	1.00	1.00	1.00
—	—	—	—
64	15	12	2
1.00	1.00	1.00	1.00
—	—	—	—
64	15	12	2
1.00	1.00	1.00	—
—	—	—	1.00
49	15	12	2

MR	LAC	HM	STG
25.0	25.0	25.0	16.7
0.107	0.091	0.097	0.086
0.47	0.44	0.07	0.75
0.40	0.56	0.64	0.25
—	—	0.29	—
—	—	—	—
0.13	—	—	—
13	16	7	4
0.69	0.72	0.79	0.67
0.22	0.20	—	0.25
0.09	0.08	0.21	0.08
—	—	—	—
16	20	7	4
1.00	1.00	1.00	1.00
—	—	—	—
18	20	7	4
1.00	1.00	1.00	1.00
—	—	—	—
20	20	7	4
0.95	1.00	1.00	1.00
0.05	—	—	—
20	19	7	4

Table 2. Continued

Locus	Allele	MDO	TEH	ECP	PM	SCI	CI	MR	LAC	HM	STG
		Populations									
IDH-2	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.86	1.00
IDH-2	B	—	—	—	—	—	—	—	—	0.14	—
Sample size (n)		5	6	7	14	10	15	20	20	7	4
MDH-1	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MDH-1	B	—	—	—	—	—	—	—	—	—	—
Sample size (n)		5	6	7	14	10	15	17	20	7	4
MDH-2	A	1.00	0.92	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00
MDH-2	B	—	0.08	—	—	—	—	—	0.05	—	—
Sample size (n)		5	6	7	14	10	15	20	20	7	4

See text for locus abbreviations. [a]Allelic frequency ≥ 0.05. —, allele, not present.

Three loci, ACOH-1, ACOH-2, and G3PDH, deviated from Hardy–Weinberg equilibrium in some populations, in all cases due to heterozygote deficiency ($P < 0.05$). ACOH-1 did not conform to Hardy–Weinberg equilibrium in the populations CV, TP, CI, MR, LAC, and HM (abbreviations are explained in Table 1). ACOH-2 was not in Hardy–Weinberg equilibrium in the populations TP, BL, GS, PM, SCI, and LAC. Finally, G3PDH deviated in 3 populations: CV, BL, and MR. No linkage disequilibria were found among alleles at any loci.

Glucose-6-Phosphate Isomerase. Significant differentiation occurred at the GPI locus between a northern (GPI allele A ≤ 0.67) and a southern group of populations (GPI allele A ≥ 0.93) (Fig. 1). Additionally, a rough north–south latitudinal cline was found in GPI (Fig. 1). The A allele increases in frequency from north to south until it becomes fixed in the MDO, PM, SCI, CI, MR, LAC, HM, and STG populations. The loci ACOH-1, ACOH-2 and GPI showed significant ($P < 0.05$) differentiation among some populations. However, ACOH-1 and ACOH-2, unlike GPI, exhibited no obvious geographic structure in their patterns of dissimilarity.

Gene Flow. F statistic results are presented in Table 3. The standard and jackknifing estimates were in good agreement with each other. For the combined populations, standard estimates of overall F, θ, and f were 0.349, 0.160, and 0.224, respectively. All were significantly >0. Overall, F, θ, and f values were 0.187, 0.044, and 0.150 for the northern group of populations (WVC, HL, DR, CV, PRC, UCF, BP, TP, BL, GS, and TEH) and were 0.421, 0.032, and 0.401 for the southern group of populations (MDO, ECP, PM, SCI, CI, MR, LAC, HM, and STG). All F statistics for both the northern and southern groups were significantly >0, except the θ estimate for the southern group.

The private alleles method provided gene flow estimates of Nm = 3.36 for all populations combined, 0.81 for the northern group, and 3.16 for the southern group. Gene flow estimates using overall θ values were Nm = 1.31 for the combined populations, Nm = 5.43 for the northern and Nm = 7.56 for the southern tick populations.

Isolation-By-Distance. Results from the isolation by distance analyses are given in Fig. 2. For all populations combined the slope was −0.911

Table 3. *F* statistics for the populations examined

Locus	F	θ	f
ACOH-1	0.389*	0.061*	0.349*
ACOH-2	0.357*	0.020	0.344*
FUMH	1.000	0.065	1.000
GPI	0.305*	0.394*	−0.147
G3PDH	1.000*	−0.026	1.000*
IDH-2	−0.018	0.003	−0.021
MDH-1	0.002	0.018	−0.017
MDH-2	−0.005	0.009	−0.014
All loci, all localities	0.349*	0.160*	0.224*
Jackknifing, all localities	0.351 ± 0.035	0.168 ± 0.134	0.244 ± 0.151
All loci, northern group	0.187*	0.044*	0.150*
Jackknifing, northern group	0.180 ± 0.178	0.047 ± 0.020	0.138 ± 0.177
All loci, southern group	0.421*	0.032	0.401*
Jackknifing, southern group	0.453 ± 0.069	0.038 ± 0.012	0.431 ± 0.064

Results are given by locus, over all loci, jackknifing over loci and standard deviations, including the locus GPI and excluding GPI. * $P < 0.05$ that F, θ and $f = 0$.

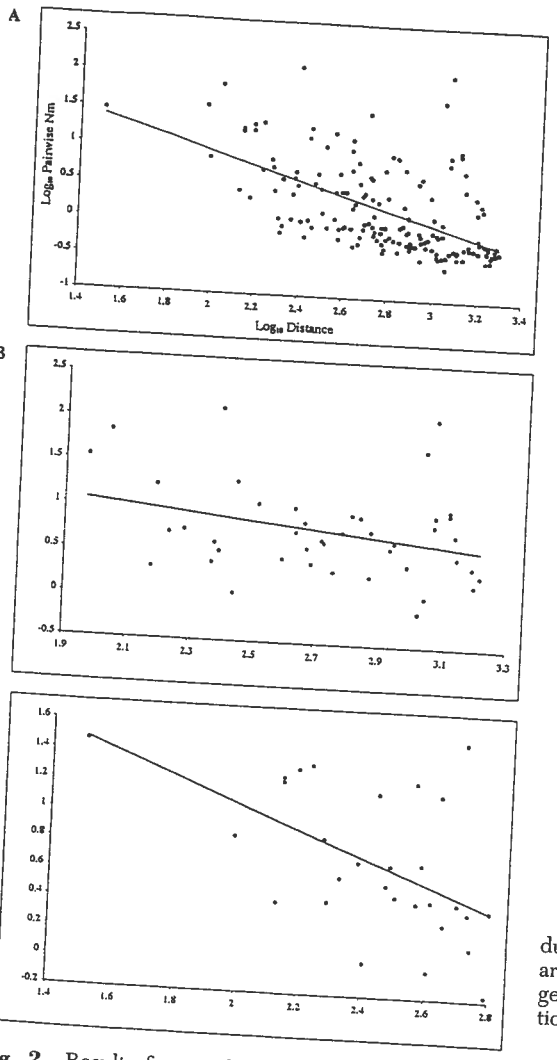

Fig. 2. Results from isolation-by-distance regression analyses. (A) All *I. pacificus* populations combined, $b = -0.911$, $R^2 = 0.28$ ($P < 0.05$), (B) Northern group of populations, $b = -0.358$, $R^2 = 0.036$ ($P = 0.118$), (C) Southern group of populations, $b = -0.840$, $R^2 = 0.229$ ($P < 0.05$).

(Fig. 2A), which is close to the slope predicted (-1.0) for the 1-dimensional stepping-stone model (Slatkin 1993). However, the regression coefficient of $R^2 = 0.28$ ($P < 0.05$) indicates that only 28% of the variation was explained by the regression. The slopes were −0.358 (Fig. 2B) and −0.840 (Fig. 2C) for the northern and southern groups, respectively. These values were below those predicted for the both the 2-dimensional (-0.5) and 1-dimensional stepping-stone models (Slatkin 1993). Regression coefficients were 0.036, $P = 0.118$ (northern group), and 0.229, $P < 0.05$ (southern group). The explained variances were only ≈4% for the northern group of populations and 23% for the southern group of populations.

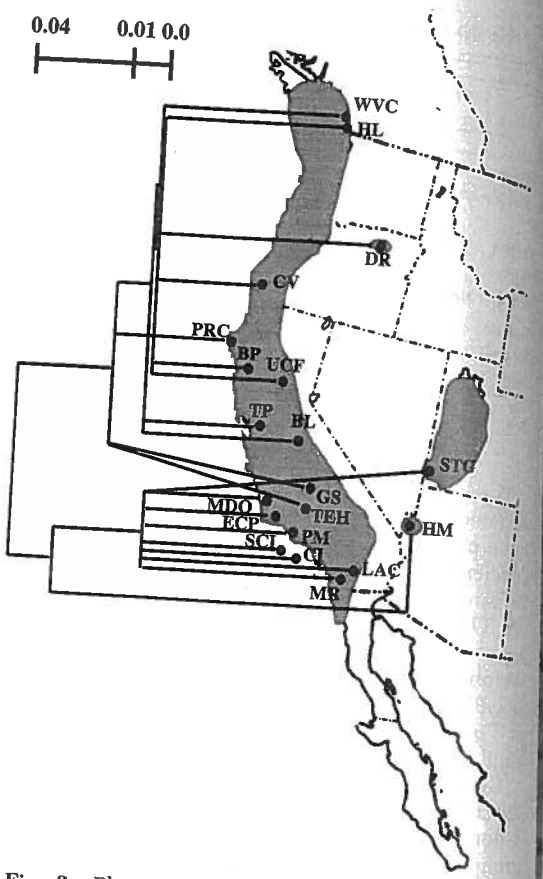

Fig. 3. Phenogram, overlain onto range map, produced from the unweighted pair group method using arithmetic averages clustering of Nei's (1978) unbiased genetic distances for the combined *I. pacificus* populations. Cophenetic correlation coefficient was 0.98.

Cluster Analyses. Within the combined populations of *I. pacificus*, overall Nei's (1978) distances ranged from 0.000 to 0.082 with a mean of 0.027. Average distance from the outgroup, *I. spinipalpis*, was 0.330. Rogers' (1972) distances ranged from 0.030 to 0.275, with distance to the outgroup averaging 0.516. A phenogram derived from the unweighted pair group method using arithmetic averages clustering of Nei's distances is shown in Fig. 3. The topology of the phenogram derived from Rogers' distances was identical to that from Nei's distance (data not shown). When all populations were combined, 2 major groupings were observed, a northern group (populations WVC, HL, DR, CV, PRC, UCF, BP, TP, BL, GS, and TEH) and a southern group (populations MDO, ECP, PM, SCI, CI, MR, LAC, HM, and STG). The cophenetic correlation coefficient for the unweighted pair group method using arithmetic averages cluster analysis using Nei's distances was 0.980. For the northern group of ticks, Nei's distance values ranged from 0.000 to 0.041 (mean = 0.008) and

for the southern group, they ra
0.029 (mean = 0.007).

Discussion

Dramatic and geographically
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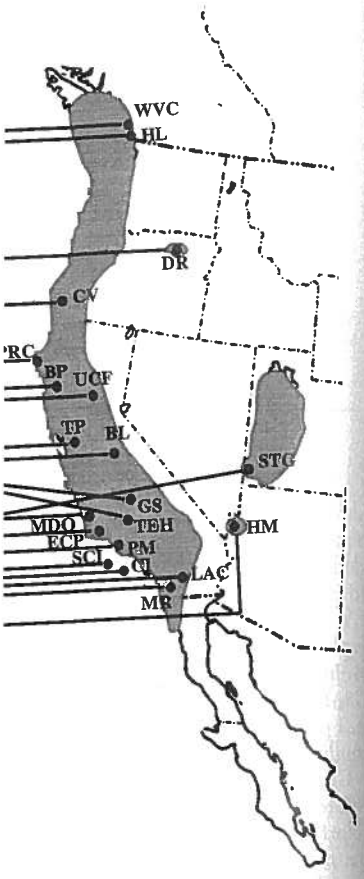

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Discussion

Dramatic and geographically structured shifts in allelic frequency were detected at the GPI locus in southern California, but the remaining loci exhibited little genetic differentiation among the various populations of *I. pacificus* surveyed. The pattern found at the GPI locus can result from any number of factors including selection, gene flow, genetic drift, historical effects, and sampling artifacts (Harrison 1990). It is, however, difficult to distinguish clinal patterns that result from recent effects such as selection and gene flow from historical effects such as secondary contact (Endler 1977). If the cline in allelic frequencies is maintained by local adaptation, then the assumption of allelic neutrality is violated for both gene flow and cluster analyses. Therefore, loci other than GPI would more accurately reflect recent gene flow and the history of *I. pacificus*. However, if the cline does not result from selection, then the genetic structure of the GPI locus could reveal important insights into migratory patterns and historical processes in *I. pacificus*.

Polymorphism. The mean percentage of polymorphic loci, 25.0, was within the range (18.9–42.9) reported before for livestock ticks (Hilburn and Sattler 1986b, Sattler et al. 1986, Hilburn et al. 1989), although it was in the lower half of the range of values. The mean average heterozygosity, 0.101, in *I. pacificus* also was comparable to the range (0.088–0.092) reported for 5 species of livestock ticks by Hilburn and Sattler (1986a, b). These values contrast with the low average heterozygosities (range, 0.016–0.025) reported for 6 species of Australian reptile ticks (Bull et al. 1984). In contrast to livestock ticks, the reduced heterozygosity in these reptile ticks was attributed to relatively small effective population sizes, and to the low vagility and solitary nature of lizard hosts (Bull et al. 1984).

The loci ACOH-1, ACOH-2, and G3PDH departed from Hardy–Weinberg equilibrium in several populations. The heterozygote deficiencies found at these loci most likely resulted from either the presence of null alleles or from scoring artifacts. The presence of null alleles (no or reduced expression) can lead to an underestimation of heterozygotes and can be troublesome to interpret without breeding studies (Murphy et al. 1996). Finally, scoring heterozygotes in ACOH-1 was especially difficult because some alleles comigrated. Under these conditions, some heterozygotes may have been scored as homozygotes.

The analysis of genic differentiation showed no particular geographic patterns except at the GPI locus. Significant genetic structure was found in central and southern California between northern and southern groups of *I. pacificus* populations. More specifically, this transitional area was just south of Tehachapi (TEH) at the southern tip of the Sierra Nevada Range and, in the coastal region, somewhere between the TP locality in the San Francisco Bay Area and the MDO locality in San Luis Obispo County (Fig. 1). If this zone represents a barrier to gene flow the reasons for its existence are not obvious. The area of genetic differentiation represents a mixture of different floristic regions none of which correlates with the observed genetic pattern (Hickman 1993). The genetic structure manifested at the GPI locus could have resulted from either recent or historical processes (see *GPI* and *Cluster Analyses* sections below).

No patterns of increased or decreased polymorphisms were seen, and no center of origin was suggested by the data. Moreover, there was no evidence for range fragmentation or founding events in the disjunct populations from the Great Basin and Range and 2 California Channel Islands because they possessed the same genetic diversity as populations in the continuous portion of the range. There was no evidence of founding events in the extremes of the range as would be found in a rapidly expanding population (Hewitt 1993).

These patterns of genetic polymorphism in *I. pacificus* suggest several distinct but not mutually exclusive explanations. Perhaps *I. pacificus* has undergone a recent range expansion or experiences high rates of gene flow or, alternatively, has persisted in the Great Basin and Range region in relatively large populations that have retained high levels of genetic polymorphism.

Glucose-6-Phosphate Isomerase. In an adaptive cline, selection can act on a single locus, whereas gene flow, drift, and historical effects could potentially affect all loci equally (Harrison 1990). Selection could explain the observed cline in *I. pacificus* because none of the other loci showed the same geographic patterns of differentiation as GPI. Latitudinal clines in GPI have been reported for other organisms including insects (Zera 1987, Van Beneden and Powers 1989, Watt et al. 1996) and temperature has been proposed to be the selective agent. No clines in allozymes have been reported previously for ticks, however (Hilburn and Sattler 1986a, b; Sattler et al. 1986; Hilburn et al. 1989; Oliver et al. 1993).

Lack of concordance among loci is not absolute evidence for maintenance of a cline by selection. The GPI cline in *I. pacificus* could have resulted easily from complex interactions among different evolutionary processes, such as secondary contact plus limited gene flow combined with stochastic effects (Harrison 1990). Further work is required to delineate the underlying causes of the allelic patterns found in GPI.

Gene Flow. Gene flow may be high even in disjunct populations of *I. pacificus*. In *Drosophila* Fallén, gene flow is considered high when Nm values are >2 (Singh and Long 1992). The significant

genetic structure at the GPI locus in southern California results in a low overall Nm estimate (1.31, F_{st} method). However, Nm estimates (F_{st} method) are high within each of the northern (5.43) and southern groups (7.56), and high using the private alleles method (3.36). If the GPI allelic pattern is not adaptive, then these estimates indicate that throughout much of its range *I. pacificus* exhibits high gene flow, except for a narrow geographic area in southern California. If the GPI cline is maintained by selection, then the genetic patterns in other loci could more accurately reflect gene flow. When GPI was removed from the analyses, we obtained estimates of Nm = 5.70 (F_{st} method) and Nm = 3.38 (private alleles), which were in accord with the foregoing higher gene flow estimates. Finally, gene flow estimates made here are low because Nm will be underestimated in populations with small sample sizes because of increased variance and the overestimation of F_{st}.

Hilburn and Sattler (1986a) suggest that broad host specificity, mobility of hosts, and anthropogenic effects (e.g., movement of livestock) contribute to the apparently high gene flow found in certain livestock ticks. For example, the lone star tick, *Amblyomma americanum* (L.), had a reported F_{st} value that provided a Nm estimate of 6.51 (Hilburn and Sattler 1986a). These same effects are likely to be important in *I. pacificus*, which parasitizes many species of birds, large mobile mammals, livestock, and companion animals (Arthur and Snow 1968, Furman and Loomis 1984). Thus, the potential for long range dispersal in this tick is great.

Isolation-By-Distance. No isolation-by-distance was found in *I. pacificus*. Slatkin (1993) predicts that a 1-dimensional stepping-stone model should produce a slope of −1.00 and a 2-dimensional model should result in a slope of −0.5. The slope (-0.91) of the isolation-by-distance regression for combined *I. pacificus* populations was near the predicted value for a 1-dimensional stepping stone model, but only 28% of the variance was explained by the regression, indicating a weak relationship between gene flow and geographic distance (Fig. 2A). The individual analyses of the northern and southern groups of *I. pacificus* also did not fit Slatkin's models (Fig. 2 B and C). Finally, an isolation-by-distance regression analysis that included only the western populations (excluding STG and HM ticks) failed to produce significant results (data not shown).

When Nm estimates are high but no distance effects are found (as seen in *I. pacificus*), populations may not be in equilibrium (Slatkin 1993). Such a situation might be found in a species undergoing a rapid range expansion (Slatkin 1993). This type of pattern has been reported for a colonizing cynipid gallwasp in Europe (Stone and Sunnucks 1993). It may be that *I. pacificus* has undergone a recent and rapid range expansion.

Finally, to detect isolation-by-distance, at least 10 polymorphic allozyme loci are normally required (Slatkin 1993). It is conceivable that the small sample size of polymorphic loci ($n = 8$) used in this study may have biased the isolation-by-distance results.

Cluster Analyses. The mean Nei's distance measure (0.027) for *I. pacificus* populations was within the range of means (0.016–0.103) reported for conspecifics in other tick species (Bull et al. 1984, Hilburn and Sattler 1986b, Oliver et al 1993). The relatively small mean Nei's distance detected in *I. pacificus* agreed with the small intraspecific distances observed in several livestock ticks. Average Nei's distances were 0.030 in *Boophilus microplus* (Canestrini) (Sattler et al. 1986), 0.006 in *A. americanum*, (Hilburn and Sattler 1986a), 0.006 in *A. cajennense* (F.), and 0.009 *A. imitator* (Kohls) (Hilburn et al. 1989). These authors suggest that high gene flow caused by host vagility and broad host range was the most important factor in preventing genetic differentiation in these livestock ticks. Broad host range and host mobility may not necessarily prevent genetic differentiation, however. In contrast, *I. scapularis*, which has a similar latitudinal range but a wider longitudinal distribution than *I. pacificus*, has a broad host range that includes bird and large mobile mammalian hosts (Lane et al. 1991), but possesses a larger mean Nei's distance (0.103) (Oliver et al. 1993).

The distance and cluster analyses support the conclusion that little genetic differentiation was found among populations of *I. pacificus*, except across a narrow geographic area in central and southern California (Fig. 3). This zone of differentiation may be the result of a barrier to gene flow, recent secondary contact, or the influence of selection at the GPI locus. When GPI was removed from the analyses, any north–south genetic structure disappeared (data not shown). Thus, GPI drives the population structure observed in *I. pacificus*, and any effort to understand the epidemiology of Lyme disease in the western North America should take into account the striking pattern of variation in GPI.

In conclusion, the lack of differentiation found in *I. pacificus* at 11 of 12 allozyme loci can be explained by either a high rate of gene flow or recent and rapid range expansion. Although the isolation-by-distance analyses suggest that a recent and rapid range expansion has occurred, a high rate of gene flow is the most acceptable explanation for the genetic patterns observed in this study. This is because of *I. pacificus'* broad host range, the high mobility of its hosts, and the high polymorphism present in disjunct populations and in populations at the extremes of the range.

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Coy (California Department of Transp
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Animal use procedures described in
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Ability of the Lyme
To Infect Rodents
(Blacklegged Tick

JOSEF

Division of Vector-Bor
Centers for I
U.S. Department of He

ABSTRACT The infect
America for mice was de
primary human-biting tic
macentor variabilis (Say)
inoculated into mice, 29
competence experiments
(North Carolina and Gec
Moreover, *I. scapularis* e
infection as nymphs. *D.*
not maintain the infectio
infection with these 2 so
iabilis were inoculated in
Larval *I. scapularis* were
larval *D. variabilis*. Altho
nymphal *D. variabilis* dic
proved to be vector com

KEY WORDS *Borreli
americanum*, Lyme disea

THERE IS LITTLE doubt that *Bo*
sensu lato Johnson, Schmid, Hy
Brenner is associated closely wi
nus *Ixodes* around the world (An
man and Gray 1994, Takada et a
et al. 1996). The principal vecto
disease are *Ixodes scapularis* Sa
Cooley & Kohls, *Ixodes persul*
Ixodes ricinus (L.). Reported isc
dorferi from other genera of ti
surfaced on an infrequent basis
clude spirochetal isolates from
tow et al. 1991), *Dermacentor* (
Walker et al. 1994,) and *Haema*
al. 1994, Banerjee et al. 1995, N
ter 1996).

For a tick to serve as an imp
burgdorferi to humans, a tick
quently feed on humans and
quiring, maintaining, and trans
chete. In the eastern U
human-biting tick species are fo
I. scapularis, *Amblyomma amu*
Dermacentor variabilis (Say).