are suspected vectors. Similarly, the claim that the newly described spirochete, *Borrelia coriaceae* is the cause of bovine epizootic abortion (BEA) in the western parts of the country, needs confirmation.

Tularemia continues to be an important health problem in the southwest-central region (Arkansas, Kansas, Louisiana, Missouri, Oklahoma, Texas) that reports 200 to 250 cases every year.

Finally, brief reference is made to tick-borne relapsing fevers, Colorado tick fever, and tick paralysis.

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CONSPECIFITY OF *IXODES SCAPULARIS* AND *IXODES DAMMINI*

Dr. Oliver received his BS degree in Biology from Georgia Southern University in 1952; MS degree, Florida State University in Zoology, 1954; PHD degree, University of Kansas in Entomology, 1962. He received an NSF postdoctoral fellowship in 1962 to study with Dr. M.J.D. White at Melbourne University in Australia, where he worked in the field of Cytogenetics. He did additional course work at the University of Maryland, Johns Hopkins University and the University of Michigan Biological Field Station. Research experience includes studies of natural history of birds, experimental work on blood flukes (schistosome worms), transmission of parasitic protozoa of snakes via mites and protozoa of deer via ticks, sensory behavior of several parasitic mites and ticks, chromosomes, sex determining mechanisms, histology and ultrastructure or arthropods, effects of gamma radiation, radiomimetic chemicals, antihormones and avermectin compounds on development and reproduction of mites and ticks, various aspects of reproductive mechanisms and strategies of parasitic arthropods including types of parthenogenesis and morphometric analysis correlated with chromosomal ploidy levels, hormones of mites and ticks, field ecology and vector aspects of ticks and spirochetes/Babesia/Theileria organisms and tick-host associations. He also spent two years in classified research in biology at Ft. Detrick, Maryland.

Reciprocal crosses between *Ixodes dammini* Spielman, Clifford, Piesman & Corwin from Massachusetts and *Ixodes scapularis* Say from Georgia produced offspring through the F3 generation when the experiment was discontinued. Reciprocal *I. dammini x Ixodes pacificus* Cooley & Kohls (California) and *I. scapularis x I. pacificus* crosses produced F1 progency; however, all progency were sterile. Assortative mating experiments between *I. dammini* and *I. scapularis* indicated that maled and females of both species mated with the

opposite sex of heterospecific or conspecific ticks when there was a choice. Conventional discrimination analysis of morphometric measurements of ticks from Georgia, North Carolina, Maryland, Massachusetts, and two populationsof F1 hybrids indicated that there were recognizable differences. However, size free (sheared) discriminant analysis indicated that these differences were largely size dependent, with much overlap of the four eastern and two hybrid populations but no overlap with *I. pacificus* from California. Analysis of chromosomes (morphology and C band) indicated no differences between the Georgia and Massachusetts populations but showed a difference between them and the California population of *I. pacificus*. Analysis of isozymes showed that the genetic identity value for the Georgia and Massachusetts populations was within the normal range for conspecific populations, whereas the California population indicated cogeneric but not conspecific relatedness laboratory conditions showed no difference in length of feeding and molting periods among Georgia, Massachusetts, and California populations. These data and results of the work of other authors on tick host preferences and vector compentence indicates that *I dammini* is not a valid species separate from *I. scapularis* Say, 1821, has priority over the name *Ixodes dammini* Spielman, Clifford, Piesman & Corwin, 1979, *I. dammini* is regulated to a junior subjective synonym of *I. scapularis* (based on Article 23 of the International Code of Zoological Nomenclature).

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EVALUATION OF A HUMAN LYME DISEASE VACCINE FOR SAFETY AND IMMUNOGENICITY

John Mays is manager of Microbiology and Research Administration at Connaught Laboratories, Inc. (CLI). A specialist in approaches to vaccine development, Dr. Mays is Team Leader for the Lyme Borreliosis Vaccine Project and was responsible for identifying the importance of using a recombinant approach to develop the vaccine. In addition to establishing the Lyme Borreliosis, Microbiology and molecular Biology research programs at CLI, Dr. Mays is responsible for coordinating the manufacturing and formulation of a (group of) meningitis vaccines that are currently in clinical trials. He was a member of the CLI Component Pertussis Project Team and the Tripedia Project Team. Tripedia, the first acellular vaccine to protect children as young as 15 months of age agains diptheria, tetanus and pertussis (DTP), received U.S. Food and Drug Administration for marketing in August 1992. Dr. Mays is a member of the American Society for Microbiology and the American Society for the Advancement of Science and has published studies and presented abstracts on a number of subjects including Lyme Disease and meningitis. Dr. Mays

Conspecificity of the Ticks *Ixodes scapularis* and *I. dammini* (Acari: Ixodidae)

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ABSTRACT Reciprocal crosses between *Ixodes dammini* Spielman, Clifford, Piesman & Corwin from Massachusetts and *Ixodes scapularis* Say from Georgia produced offspring through the F_3 generation when the experiment was discontinued. Reciprocal *I. dammini* × *Ixodes pacificus* Cooley & Kohls (California) and *I. scapularis* × *I. pacificus* crosses produced F_1 progeny; however, all progeny were sterile. Assortative mating experiments between *I. dammini* and *I. scapularis* indicated that males and females of both species mated with the opposite sex of heterospecific or conspecific ticks when there was a choice. Conventional discriminant analysis of morphometric measurements of ticks from Georgia, North Carolina, Maryland, Massachusetts, and two populations of F_1 hybrids indicated that there were recognizable differences. However, size-free (sheared) discriminant analysis indicated that these differences were largely size-dependent, with much overlap of the four eastern and two hybrid populations but no overlap with *I. pacificus* from California. Analysis of chromosomes (morphology and C band) indicated no differences between the Georgia and Massachusetts populations but showed a difference between them and the California population of *I. pacificus*. Analysis of isozymes showed that the genetic identity value for the Georgia and Massachusetts populations was within the normal range for conspecific populations, whereas the California population indicated congeneric but not conspecific relatedness to the Georgia and Massachusetts populations. Life cycle data collected under similar laboratory conditions showed no differences in length of feeding and molting periods among Georgia, Massachusetts, and California populations. These data and results of the work of other authors on tick host preferences and vector competence indicate that *I. dammini* is not a valid species separate from *I. scapularis*. Because the name *Ixodes scapularis* Say, 1821, has priority over the name *Ixodes dammini* Spielman, Clifford, Piesman & Corwin, 1979, *I. dammini* is relegated to a junior subjective synonym of *I. scapularis* (based on Article 23 of the *International Code of Zoological Nomenclature*).

KEY WORDS *Ixodes scapularis*, *Ixodes dammini*, Lyme disease

LYME DISEASE ACCOUNTED for 81% of all reported cases of arthropod-transmitted diseases in the United States during 1986–1990, and there were 9,344 Lyme disease cases in 1991 (Centers for Disease Control 1992). Moreover, because physicians do not always report all Lyme disease cases, the number is likely to be greater than statistics indicate. The etiologic agent that causes Lyme disease is the spirochete *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner (Burgdorfer et al. 1982, Johnson et al. 1984). Reports indicate that the principal tick vectors of Lyme disease in the northeastern and northcentral United States are *Ixodes dammini* Spielman, Clifford, Piesman & Corwin, and in the western United States, *Ixodes pacificus* Cooley & Kohls (review by Lane et al. 1991). The proven laboratory experimental vector (Burgdorfer & Gage 1986, Piesman & Sinsky 1988) and presumed natural vector in the southern United States is the black-legged tick, *Ixodes scapularis* Say.

Before the late 1970s, *I. dammini* was not recognized as distinct from *I. scapularis*; the geographic range of *I. scapularis* was reported to be roughly the eastern half of the United States, although it was not evenly distributed (Keirans & Clifford 1978). Subsequently, *I. dammini* was described as a new species distinct from the more southerly distributed *I. scapularis* (Spielman et al. 1979). Previous records of *I. scapularis* in the northeastern United States were interpreted as incorrect and thought to represent either *Ixodes muris* Bishopp & Smith or the heretofore unrecognized *I. dammini*. The description of *I. dammini* was based primarily on external morphological characters of each developmental stage and sex. The described characters thought to be most significant in separating *I. dammini* from *I. scapularis* occur in the nymphal stage, although differences were reported in all tick stages. In the species description, photographs of certain morphological characters of ticks from Nantucket Island, MA, and ticks from Savannah, GA, did indeed show some differences, but lack of quantitative data and stated range of morphological variation in different geographic populations were worrisome. Moreover, field-collected specimens from various geographic areas frequently do not exhibit such clear differences. Characters of specimens are often intermediate between the published descriptions of *I. dammini* and *I. scapularis* and raise doubts about the validity of *I. dammini* as a separate species. Nevertheless, the new species was generally accepted by the biomedical community, and until now, *I. dammini* has been regarded as the single most important vector of the Lyme disease agent in North America (Lane et al. 1991).

The belief that *I. dammini* does not occur south of Maryland and that *I. scapularis* is a separate and distinct species yet unproven as a natural vector of Lyme disease has caused delays in Lyme disease surveillance in the South. The general attitude among physicians and veterinarians has been that Lyme disease is not a problem in that area, although patients present clinical symptoms of it. We propose to demonstrate that *I. dammini* is not distinct from *I. scapularis* and that the two species should be synonymized. Ticks identified as *I. dammini* should be referred to as *I. scapularis*, based on Article 23 of the *International Code of Zoological Nomenclature*.

Materials and Methods

Ticks. Laboratory colonies of *I. scapularis* (from Statesboro, Bulloch County, GA), *I. dammini* (from Great Island, West Yarmouth, Cape Cod, MA) and *I. pacificus* (from Point Reyes, Marin County, CA) were established. Immature ticks were fed on laboratory white mice (*Mus musculus*) and adults were fed on New Zealand white rabbits (*Oryctolagus cuniculus*). When not feeding, ticks were maintained at 97–100% RH (Winston & Bates 1960) with a 14:10 (L:D) h photoperiod at 25–27°C. Various life cycle parameters were recorded, including fecundity and fertility. These are presented, but other measurements will be reported elsewhere.

Hybridization. Reciprocal crosses were attempted using *I. scapularis* × *I. dammini*, *I. scapularis* × *I. pacificus*, and *I. dammini* × *I. pacificus*. Virgin males and females of similar ages were placed together in vials for 24 h before being placed on New Zealand white rabbits to feed and continue mating. Each group consisted of 10 males and 10 females. The interspecific pairs of ticks were placed on one ear and an intraspecific control group on the other ear of each rabbit. Subsequent to engorgement, ticks were monitored for fecundity and fertility and, if progeny were produced, they were also analyzed. Statistical analysis of fertility data was performed after transforming each percentage to its corresponding arcsine square root.

Assortative Mating. Experiments were designed to allow *I. scapularis* and *I. dammini* females and males to mate with conspecific and heterospecific mates. Part 1 of the experiment involved four replicates of 10 females placed with 10 marked heterospecific and 10 marked conspecific males. Part 2 consisted of four replicates of 10 males with 10 heterospecific and 10 conspecific females. Controls for both parts consisted of 10 conspecific pairs of each species. Vials containing the controls and experimental ticks were examined at 4-h intervals for the first 48 h and daily thereafter. Mating pairs were removed to individual vials and monitored daily for mating separation, remating, and death. Females and males were dissected for presence of sperm upon dying or at the end of the experiments.

Morphometrics. Multivariate morphometric analyses were conducted using ticks of the first (or later) laboratory generation originating from four different geographic regions, and two populations of F_1 hybrids produced by reciprocal crosses between the Georgia and Massachusetts populations. The four geographic areas included the aforementioned Georgia and Massachusetts groups and one population each consisting of first laboratory generation ticks from Cape Hatteras, Dare County, NC, and Assateague Island, Worcester County, MD. We measured 17 female, 25 male, and 23 nymphal characters, including lengths and widths of diagnostic characters used to separate *I. dammini* from *I. scapularis*. Characters selected for morphometric analyses included those distances consistent with published described values for *I. dammini* and *I. scapularis* (Cooley & Kohls 1945, Keirans & Clifford 1978, Spielman et al. 1979). In addition, we included measurements that were previously represented on a relative basis (smaller, larger, etc.), including length and width of coxae and internal coxal spurs, distance between cornua "tips," distance between auriculae "tips," and length of the internal denticle file of the hypostome. Some characters, such as hypostome tip (pointed or rounded), auriculae length, and median plate punctations, proved highly variable within groups or were difficult to measure consistently; these were excluded. Care was taken to avoid "weighting" character suites, usually caused by duplications of measured distances.

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Persons interested in the complete list of 65 characters measured may contact H.J.H.

Bilateral measurements were averaged, and all data were transformed to natural logarithms to equalize variances. Size-in and size-free (sheared) discriminant analyses (DA) were done using a Digital Equipment Corporation VAX minicomputer running SAS GLM statistical software (SAS Institute 1990). Size-free DA involved principal components analysis using a covariance matrix, comparison of principal component 1 (PC1) with variables to confirm positive or zero correlation, regression of data against PC1 to remove "size," and DA of residuals.

Chromosomes. All chromosome observations were made on gonads of at least 20 engorged nymphs of each species, 4–10 d after feeding. Gonads were dissected in Shen's saline (9 g NaCl, 0.42 g KCl, 0.25 g $CaCl_2$, 1 liter H_2O) (Oliver & Bremner 1968). Dissected tissues were placed on a coverslip and were subsequently stained with 2% lacto-aceto-orcein (Breland 1961) and squashed or Giemsa stained. Tissues to be stained with Giemsa were transferred to a drop of hypotonic solution (0.45% sodium citrate) for 15 min, covered with a piece of cellophane (1 × 1 cm) and squashed. The coverslip was immersed in fresh Carnoy's fixative for 15 min at room temperature, allowed to dry, covered with Giemsa solution for 30 min before rinsing in distilled H_2O, then air dried. The C-banding procedure was based on modifications of Arrighi & Hsu (1970) and Sumner (1972).

Karyotypes were determined by arranging chromosomes in order of decreasing lengths, matching them according to lengths and centromere positions, then numbering them. Chromosomes were photographed using Kodak Technical Pan 2415 film and printed on Kodak Kodabromide FS paper. Prints were used for chromosome measurements and comparisons. Relative chromosome lengths were the quotients of the length of individual chromosomes divided by total length of all chromosomes in a haploid set, including the one being measured (Short et al. 1989). Comparisons among measurements of relative lengths of chromosomes were made by analysis of variance (ANOVA) and Tukey's HSD multiple comparison tests. Significance was measured at the 5% level (Zar 1984). Amount and distribution of heterochromatin were judged by visual inspection.

Isozymes. Male and female ticks from laboratory colonies of *I. dammini*, *I. scapularis*, or *I. pacificus*, live or stored frozen at −70°C, were homogenized individually in 6 μl (males) or 10 μl (females) of a 0.01*M* Tris·HCl, 0.001 *M* EDTA, 10% sucrose, 1% Triton-X 100, pH 7.0 buffer. After centrifugation, 2-μl samples of the supernatant were added to wells in vertical-slab 5% polyacrylamide gels containing either a 0.1 *M* Tris, 0.02 *M* borate, 0.0015 *M* EDTA, pH 8.5 buffer (TBE) or a 0.022 *M* Tris, 0.007 *M* citric acid, pH 7.1 buffer (TC). The TBE tank buffer contained the same concentration of buffer components as the gel, and the TC tank buffer contained half the concentration of buffer components. Gels had been prerun for 1 h at 100 V. Samples were electrophoresed at 300 V for 3 h at 4°C. TC tank buffer was replaced after 2 h. Gels were stained for enzyme activity using standard techniques (Shaw & Prasad 1970, Harris & Hopkinson 1977, Steiner & Joslyn 1979, Pasteur et al. 1988) as modified by Munstermann (1979).

Table 1. Hybridization of *I. scapularis* and *I. pacificus*

Cross[a]	Fecundity		% Fertility	
	No. ♀♀	Mean ± SE	No. ♀♀	Mean ± SE
I.s. ♀ × *I.s.* ♂	19	860 ± 154	19	84.8 ± 4.8
I.p. ♀ × *I.p.* ♂	11	379 ± 103	9	50.9 ± 8.0
I.s. ♀ × *I.p.* ♂	3	1870 ± 555	3	52.7 ± 13
F_1sp ♀ × F_1sp ♂	7	273 ± 92.2	7	0
I.p. ♀ × *I.s.* ♂	7	462 ± 116	1	68.3
F_1ps ♀ × F_1ps ♂	6	120 ± 16	6	0

[a] *I.s.*, *I. scapularis*; *I.p.*, *I. pacificus*. In hybrid crosses, female parent is listed first; i.e., F_1sp is progeny from the cross female *I. scapularis* × male *I. pacificus*.

Results

Hybridization. Reciprocal crosses using *I. scapularis* × *I. pacificus* and *I. dammini* × *I. pacificus* resulted in mating, engorgement of females, oviposition, and production of F_1 hybrids. Hybrids were reared to adults and crossed among themselves (sib matings) and back-crossed with parent species. Matings were successful, but fertility was zero among F_1 sib-mating pairs (Tables 1 and 2) and zero, or (among four pairs) practically zero, among back-crosses (Table 3). Reciprocal crosses between *I. scapularis* and *I. dammini* and their F_1 and F_2 progeny were fully fertile (Table 4). Comparison of fertility among the three parent laboratory colonies of *I. scapularis*, *I. dammini*, and *I. pacificus* indicated a highly significant difference among the groups ($F = 9.675$, $P = 0.0006$). Scheffé's multiple comparison *F* test showed no difference between *I. scapularis* and *I. dammini* but a significant difference ($P < 0.05$) between *I. scapularis* and *I. pacificus* and between *I. dammini* and *I. pacificus*. There was no significant difference ($F = 0.885$, $P = 0.5264$) in fertility among the *I. scapularis* and *I. dammini* parent colonies and the F_1 and F_2 hybrids (Table 4).

Table 2. Hybridization of *I. dammini* and *I. pacificus*

Cross[a]	Fecundity		% Fertility	
	No. ♀♀	Mean ± SE	No. ♀♀	Mean ± SE
I.d. ♀ × *I.d.* ♂	10	790 ± 217	5	89.8 ± 3.6
I.p. ♀ × *I.p.* ♂	11	379 ± 103	9	50.9 ± 8
I.d. ♀ × *I.p.* ♂	3	679 ± 77	2	63.3 ± 14
F_1dp ♀ × F_1dp ♂	5	193 ± 27	5	0
I.p. ♀ × *I.d.* ♂	2	209 ± 230	2	21.3 ± 8[b]
F_1pd ♀ × F_1pd ♂	5	213 ± 92	5	0

[a] *I.d.*, *I. dammini*; *I.p.*, *I. pacificus*. In hybrid crosses, female parent is listed first; i.e., F_1dp is progeny from the cross female *I. dammini* × male *I. pacificus*.
[b] Eggs were contaminated with mold.

Table 3. Backcrossing F_1 × parents, *I. scapularis*, *I. dammini*, and *I. pacificus*

Cross[a]	Fecundity		% Fertility	
	No. ♀♀	Mean ± SE	No. ♀♀	Mean ± SE
I.p. ♀ × F_1pd ♂	3	125 ± 71.5	3	0
F_1pd ♀ × *I.p.* ♂	4	1022 ± 399	4	0.3[b]
I.d. ♀ × F_1dp ♂	3	830 ± 738	3	0.2[b]
F_1dp ♀ × *I.d.* ♂	8	1169 ± 375	8	0
I.p. ♀ × F_1ps ♂	5	241 ± 124	5	0.05[b]
F_1ps ♀ × *I.p.* ♂	8	750 ± 241	8	0.02[b]
F_1sp ♀ × *I.s.* ♂[c]	4	380 ± 106	4	0

[a] *I.d.*, *I. dammini*; *I.p.*, *I. pacificus*; *I.s.*, *I. scapularis*. In hybrid crosses, female parent is listed first; i.e., F_1sp is progeny from the cross female *I. scapularis* × male *I. pacificus*.
[b] A few larvae hatched from one female in each group.
[c] No data for reciprocal cross (female *I. scapularis* × male F_1sp).

Fecundities among *I. scapularis* × *I. pacificus* F_1 sib crosses were low (means,120 and 273 eggs) (Table 1) as were *I. dammini* × *I. pacificus* crosses (means, 193 and 213 eggs) (Table 2) when compared with fecundities of *I. scapularis* (mean, 860 eggs) and *I. dammini* (mean, 790 eggs), but not as low when compared to *I. pacificus* (mean, 379 eggs). Fecundity of back-crosses of F_1 × parent species were variable (Table 3). When superficially comparing fecundity among *I. scapularis* × *I. dammini* crosses and their hybrids, there appeared to be a slight reduction in fecundity of the inbred F_2 females (Table 4). Indeed, an ANOVA confirmed a difference ($F = 3.172$, $P = 0.007$) in mean fecundities among all groups. However, only the *I. dammini* × *I. scapularis* cross and the F_2 sd (*scapularis* female × *dammini* male) cross had significantly different fecundities (Scheffe's multiple comparison *F* test, $P < 0.05$); the *I. dammini* × *I. scapularis* group had the highest fecundity (mean, 1731) and the F_2 sd group the lowest (mean, 314) (Table 4). Fecundities of none of the crosses was significantly different from those of the two parent colonies (*I. scapularis* and *I. dammini*). Interestingly, the ANOVA of mean fecundities among laboratory colonies of the three groups (*I. scapularis*, *I. dammini*, *I. pacificus*) showed no significant difference ($F = 2.313$, $P = 0.1131$).

Table 4. Hybridization data for *Ixodes* colonies and *I. scapularis* × *I. dammini* crosses

Mated ♀♀ (species/cross)[a]	Fecundity		% Fertility	
	No. ♀♀	Mean ± SE	No. ♀♀	Mean ± SE
I.s. colony	19	860 ± 154	19	84.8 ± 4.8
I.d. colony	10	790 ± 217	5	89.8 ± 3.6
I.p. colony	11	379 ± 103	9	50.9 ± 8.0
I.d. × *I.s.*	5	1731 ± 370	5	97.1 ± 0.5
F_1 *ds* × F_1 *ds*	1	764	1	94.1
F_2 *ds* × F_2 *ds*	7	468 ± 149	4	86.0 ± 4.4
I.s. × *I.d.*	8	948 ± 66	8	93.2 ± 4.5
F_1 *sd* × F_1 *sd*	5	950 ± 175	4	83.1 ± 6.4
F_2 *sd* × F_2 *sd*	7	314 ± 77	5	70.5 ± 7.7

First species listed in all crosses is the female; same sequence applies in hybrid crosses; i.e., F_1 *ds* is progeny from cross female *I. dammini* × male *I. scapularis*.
[a] *I.s.*, *I. scapularis*; *I.d.*, *I. dammini*.

Table 5. Assortative mating of *I. scapularis* and *I. dammini*

Group[a]		Matings		
Species	Limited Sex	Conspecific	Heterospecific	Not mated
I. dammini ♀	40	16	19	5
I. dammini ♂	40	14[b]	4	22
I. scapularis ♀	40	25[b]	10	5
I. scapularis ♂	40	12	24[b]	4

Successful mating determined by presence of spermatophore in female.
[a] Each group consisted of four replicates of 10 of the limited sex and 10 conspecific and 10 heterospecific opposite sex.
[b] Significantly different ($P < 0.05$, Student's *t* test with isolation index [Malogolowkin-Cohen et al. 1965]).

Assortative Mating. When given a choice of mates, female *I. dammini* did not show a preference between conspecific and heterospecific males, but female *I. scapularis* preferred conspecific males. Male *I. dammini* preferred conspecific females, whereas male *I. scapularis* preferred heterospecific *I. dammini* females (Table 5). These experiments showed that there was no barrier to mating between the Georgia and Massachusetts ticks even when potential mates from their geographic populations were available. Moreover, even in the two experiments that showed preference for conspecific mates, interspecific matings occurred.

Morphometrics. Although *I. dammini* males were described as having the greatest number of diagnostic characters separating them from *I. scapularis* (Spielman et al. 1979), individuals among the different geographic groups were virtually indistinguishable. Moreover, they were the most variable when compared with females and nymphs. The first two principal components (PCs) were responsible for 58 and 13% in females and 52 and 16% in nymphs, respectively, whereas the variance in males was more equally distributed among the remaining PCs; in males, PCs 1 and 2 were responsible for only 32 and 17% of the total variance. In females, the internal spur on coxae I was used in the description of *I. dammini* as one of two diagnostic characters separating *I. scapularis* from *I. dammini*. This character was the most strongly correlated with general size, suggesting it is size- and perhaps environmentally dependent. The other character

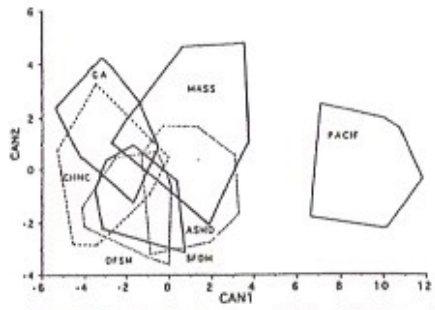

Fig. 1. Scatterplot showing size-free discriminant analysis (DA) of geographic populations of *I. scapularis*, *I. dammini*, *I. pacificus* and laboratory hybrids. CAN1 and CAN2, canonical scores (discriminant functions) one and two, respectively; ASMD, *I. dammini*, Assateague Island, Worcester County, MD (n = 200); CHNC, *I. scapularis*, Cape Hatteras, Dare County, NC (n = 149); DFSM, F_1 hybrids, from *I. dammini* female × *I. scapularis* male (n = 39); GA, *I. scapularis*, Bulloch County, GA (n = 41); MASS, *I. dammini*, Great Island, MA (n = 59); PACIF, *I. pacificus*, Sonoma County, CA (n = 50); SFD, F_1 hybrid from *I. scapularis* female × *I. dammini* male (n = 40).

used to separate females of these species is based on differences in auriculae. Differences in this character among groups appear to be less distinct and highly variable within groups. The progeny of *I. scapularis* females and *I. dammini* males were more similar to groups from North Carolina and Georgia than were the progeny of the reciprocal cross. This suggests a possible maternal effect.

Of the stages studied, nymphs demonstrated the most distinct morphological differences among the included geographic populations, laboratory colonies, and laboratory hybrids. Although they were compared on a multivariate level, characters associated with the basis capitulum showed the most significant differences ($P < 0.01$). Conventional (size-in) discriminant analysis (DA) suggested recognizable differences, but sheared (size-free) DA indicated these differences were largely size-dependent. There was much overlap of the four eastern and two hybrid groups but none with nymphal *I. pactificus* from California (Fig. 1). To insure that inclusion of *I. pacificus* in analyses did not cause the remaining groups to overlap, we performed an alternate sheared DA on adults and nymphs with similar degrees of overlap and arrangement in morphological space (figure not shown).

Chromosomes. Chromosome analysis of *I. scapularis*, *I. dammini*, and *I. pacificus* revealed no gross differences among the three species. All share the same chromosome number ($2n$ = 28), sex chromosome mechanism (XX female, XY male), and relative lengths of chromosomes at mitotic metaphase. Moreover, there are no consistent differences between the C bands of chromosomes of the three except that *I. scapularis* and *I. dammini* share an additional interstitial C band in chromosome 7, which is lacking in *I. pacificus*.

Isozymes. Enzyme allele frequencies for seven polymorphic loci of *I. scapularis*, *I. dammini*, and *I. pacificus* were analyzed. They included adenylate kinase (AK), fumarate hydratase (FUM), glucose phosphate isomerase (GPI), two forms of glycerol-3-phosphate dehydrogenase (GPD), hexokinase (HK), and isocitrate dehydrogenase (IDH). Although resolution of additional enzymes is required to define more precisely the genetic relatedness of *I. scapularis* and *I. dammini*, a similarity matrix using Nei's genetic identity (Nei 1972) with respect to the seven loci indicates that *I. scapularis* and *I. dammini* are closely related to each other. In fact, the genetic identity value (0.902) for these species is within the normal range for conspecific populations (Ayala et al. 1974, Avise 1975). "If similarities between populations are arranged on a scale from 0 to 1, with 1 indicating genetic identity, conspecific populations usually fall above 0.85." (Avise 1975). The corresponding Nei's genetic distance (0.103) further indicates limited evolutionary divergence between *I. scapularis* and *I. dammini*. Additionally, neither species-diagnostic nor species-discriminatory enzyme phenotypes were identified. Neither *I. scapularis* nor *I. dammini* showed close relatedness to *I. pacificus*. The pairwise comparisons of Nei's genetic identity between *I. scapularis* and *I. pacificus* (0.306) and *I. dammini* and *I. pacificus* (0.403) fall within the normal range of congeneric populations, whereas comparisons between *I. dammini* and *Dermacentor variabilis* (0.09) and *I. scapularis* and *D. variabilis* (0.0) do not (Ayala et al. 1974, Avise 1975).

Life Cycles. There appear to be no consistent differences in most life cycle parameters that would convincingly refute conspecificity between *I. scapularis* and *I. dammini* when reared under similar laboratory conditions. However, as noted in the section on hybridization, ANOVA showed a significant difference in fertility among *I. scapularis*, *I. dammini*, and *I. pacificus* laboratory colonies (F = 9.675, P = 0.006). Scheffe's multiple comparison F test indicated no difference between *I. scapularis* and *I. dammini*, but significant differences ($P < 0.05$) between both *I. scapularis* and *I. pacificus* and *I. dammini* and *I. pacificus;* no difference in fertility was found among *I. scapularis*, *I. dammini*, and F_1 and F_2 hybrids (Table 4). Although fecundity varied intra- and interspecifically among *I. scapularis* (mean, 860), *I. dammini* (mean, 790), and *I. pacificus* (mean, 379), ANOVA indicated no significant difference among the three species. The variation of fecundity within each group was as great as or greater than the variation among the species. Additional data on various parameters of laboratory life cycles are being investigated.

Discussion

The question of accurate identity of Lyme disease vectors is of more than academic biosystematic interest. It is essential to know the correct identity of the primary vectors of *Borrelia burgdorferi* in all parts of the United States. The large number of human cases of Lyme disease makes vector identification a medical necessity, especially in view of the difficulty of rapid clinical diagnosis of this disease.

The description of *I. dammini* as a new species stimulated questions as to whether there also might be biological differences between *I. dammini* and *I. scapularis;* e.g., different tick host preferences (especially among the immature stages), tick developmental differences, and different vector competencies between the two tick species. If such differences are present, they may well affect the natural enzootiology of the Lyme disease spirochete and consequently the risk of infection to humans. Because *I. dammini* was thought to be restricted to the northern United States, there were questions whether Lyme disease would ever be more than a medical curiosity in the South, especially because at that time it was not known whether *I. scapularis* was an efficient vector of *B. burgdorferi*. If *I. scapularis* was not an efficient vector, or if it also demonstrated other critical differences, people in the South had little reason to fear contracting Lyme disease. Unfortunately, there were repeated instances of southerners presenting clinical symptoms of Lyme disease. Accepted dogma is that Lyme disease is not a serious problem in the southeastern United States. This misconception continues among some scientists and physicians, even though human cases (as defined by CDC case definitions) are diagnosed in this region. Indeed, 715 human Lyme disease cases were reported in Georgia during 1989 (Centers for Disease Control 1990), although there is some question about the validity of the number of cases (Kaslow 1992).

The strongest evidence that *I. dammini* is not a different species from *I. scapularis* is obtained from data on hybridization and assortative mating. These data are supported by studies of multivariate morphometric analysis, chromosomes, isozymes, and laboratory life cycles. Further support for conspecificity is derived from published results on tick host preferences (James & Oliver 1990), published (Burgdorfer & Gage 1986, Piesman & Sinsky 1988) and unpublished (W. Burgdorfer, personal communication) observations on vector competencies, and ribosomal DNA sequences of several geographic populations of *I. scapularis*, *I. dammini*, and *I. pacificus* (D. Wesson et al., unpublished data). DNA sequencing of ITS 1 and ITS 2 of rDNA suggests that *I. scapularis* and *I. dammini* are a single species in which even geographically isolated populations are very similar and distinct from *I. pacificus*.

Hybridization and Assortative Mating. As noted in the Results section, reciprocal crosses were made between *I. pacificus* from California, *I. scapularis* from Georgia, and *I. dammini* from Massachusetts. Results of these experiments indicated that *I. pacificus* is genetically incompatible with *I. scapularis* and *I. dammini* as demonstrated by sterile F_1 hybrids. *I. scapularis* and *I. dammini* are genetically similar as shown by fertile hybrids. The latter crosses were discontinued after third-generation hybrid adults were produced. Although there was no reluctance to mate and no genetic incompatibility between ticks of the Georgia (*I. scapularis*) and Massachusetts (*I. dammini*) populations, experimental design allowed no choice regarding selection of mates between each geographic population. Thus, it could be argued that had such a choice been available, ticks from each region would have selected mates from their own populations (conspecific) in preference to the other (heterospecific). If such assortative matings occurred, then there might be reproductive isolation of ticks from Georgia and Massachusetts even if representatives of each population were to come together in nature. Thus, it is of interest to know whether there is a preference for mates from one's own geographic region when given a choice. To answer this question, assortative mating experiments were conducted.

Experimental results (Table 5) show that not only are the *I. scapularis* ticks from Georgia and *I. dammini* ticks from Massachusetts genetically compatible, they also readily mate with each other in the presence of potential mates from their own region. Thus, should ticks from these two populations come together in nature by population overlap or transport by hosts, the ticks would readily mate and produce viable progeny.

Morphometrics. Multivariate statistics linearly combine values and compare entire morphological character suites simultaneously, thus they may compensate for intercorrelations among individual variables otherwise neglected by univariate ANOVA (Willig & Owen 1987). Discriminant function (DA) and principal component (PC) analyses are two of the most widely used multivariate methods (Marcus 1990). To equalize variances among groups, raw data may be transformed to natural logarithms (Delfinado-Baker and Houck 1989). Multivariate statistical procedures that partition size as a variable appear to be the most accurate means to compare patterns of character variation and covariation among geographic populations. Structures are composed of two major factors: size and shape

(Bookstein et al. 1985). Variation in size may be related to temporal, environmental, or ontogenetic factors and may be responsible for morphological variation within or among groups, thus having a confounding effect on conventional morphometric analyses used to evaluate differences among geographic populations (Humphries et al. 1981, Strauss 1985, Rohlf & Bookstein 1987). Results of sheared DA are intended to be compared with, as opposed to replacing, results of conventional DA (Bookstein 1989, Somers 1989, Sundberg 1989); therefore distinctions in morphological space illustrated by conventional DA scatterplots of nymphs may prove useful to identify geographic populations.

Morphological characters of *I. scapularis* and *I. dammini* vary among individual specimens in nature. Of the nine characters used in the species description to distinguish *I. dammini* from *I. scapularis* (Spielman et al. 1979), five were relative and not measured (auriculae, hypostome apex, coxae I internal spurs, median plate setae, goblets); four were measured characters (palpal L:W ratio, internal denticle file, spiracular plate length, median plate punctations). We measured characters that had not been measured but used as relative characters (smaller, larger, etc.) in the species description of *I. dammini*. In addition, a much larger number of characters (17 female, 25 male, 23 nymphal) were analyzed during our investigation. Interestingly, when size-in discriminant analysis was evaluated, nymphs from Massachusetts could be recognized as morphologically different from those from Georgia, but size-free discriminant analysis indicated these differences were size-dependent. This size difference may be caused by different environmental selection pressures of northern and southern climates. The scatterplot (Fig. 1) of size-free discriminant analysis of ticks from Massachusetts, Maryland, North Carolina, Georgia, California, and reciprocal F_1 hybrids of Massachusetts × Georgia clearly shows that *I. pacificus* from California is morphologically distinct from the eastern United States populations and that there is overlap of morphological characters among the eastern populations. The data suggest a north-south cline of a single species along the Atlantic Coast and further support the conclusion drawn from the hybridization and assortative mating experiments that there are no consistent recognizable differences between *I. scapularis* and *I. dammini*.

Chromosomes and Isozymes. Chromosome analysis of ticks from California (*I. pacificus*), Georgia (*I. scapularis*), and Massachusetts (*I. dammini*) indicated great similarity among karyotypes. The one consistent difference noted was an additional interstitial C band in chromosome 7 of *I. scapularis* and *I. dammini*, which was absent in *I. pacificus*. These chromosome data indicate that *I. scapularis* and *I. dammini* are similar, whereas *I. pacificus* is different.

As noted in the Results section, isozyme analysis of *I. scapularis*, *I. dammini*, and *I. pacificus* indicates that the genetic identity value (0.902) for *I. scapularis* and *I. dammini* is within the normal range for conspecific populations; the values for *I. scapularis* and *I. pacificus* (0.306) and for *I. dammini* and *I. pacificus* (0.403) are not within conspecific range but are within congeneric range (Ayala et al. 1974, Avise 1975). Interestingly, several enzymes distinguished *I. pacificus* from either *I. scapularis* or *I. dammini*, including FUM, GPI, GPD, and esterases.

Life Cycles. It was reported that there was a marked difference in nymphal molting times between *I. scapularis* and *I. dammini* (Krinsky 1979). This alleged difference was based on laboratory rearing data of *I. dammini*, which were compared with published data on *I. scapularis*. Such a difference has not been confirmed. Indeed, our data collected under similar laboratory conditions show molting times of nymphal *I. scapularis*, *I. dammini*, and *I. pacificus* to be similar. There were differences ($P < 0.05$) in fertility between *I. scapularis* and *I. pacificus* and *I. dammini* and *I. pacificus*, but not between *I. scapularis* and *I. dammini*.

Host Preference. *Ixodes scapularis* and *I. dammini* feed on a wide variety of animals. Adults feed most commonly on large mammals, especially white-tailed deer, *Odocoileus virginianus* (Zimmermann) (Lane et al. 1991). In nature, the white-footed mouse, *Peromyscus leucopus* (Rafinesque), is the principal host of immature *I. dammini* (Lane et al. 1991). *I. scapularis* immatures parasitize small mammals, birds and reptiles, especially lizards (Bishopp & Trembley 1945; Rogers 1953; J.H.O. & Gregory A. Cummins, unpublished data). Before the laboratory study on feeding and host preference of immature *I. scapularis*, *I. dammini*, and *I. pacificus* was done (James & Oliver 1990), it was presumed that differential host preference of immatures was additional support for the separation of *I. dammini* and *I. scapularis* as distinct species. Now it is clear that, at least in the laboratory, *I. scapularis*, *I. dammini*, and *I. pacificus* cannot be distinguished from each other based on larval and nymphal feeding success or preference for mice, chickens, and lizards (James & Oliver 1990). More larvae of all three tick species fed on mice than lizards and more on lizards than chickens. In host choice experiments, they preferred mice, lizards, and chickens in that order, except for *I. pacificus* larvae, which showed no significant difference ($P > 0.05$) in preference between mice and chickens. Nymphs of the three species showed no significant difference in feeding success or host preference between mice and lizards. More nymphs fed on mice than on chickens when placed on hosts, and they preferred mice to chickens, except for *I. dammini* nymphs, which showed no preference between hosts. More nymphs fed on lizards than chickens and chose them except for nymphal *I. pacificus*, which showed no preference. Larvae and nymphs of all three species fed for longer periods on lizards than on mice or chickens; they typically fed for 3–5 d on mice and chickens and for 7–13 d on lizards. Thus, in nature, perhaps one reason immatures may be detected more often on lizards than on homeotherms is because they remain on them approximately 3 times longer.

Vector Competence for the Lyme Disease Spirochete. Published reports of vector competence experiments in the laboratory indicated that *I. scapularis* was a fully competent vector of *B. burgdorferi* (Burgdorfer & Gage 1986, Piesman & Sinsky 1988). Although no rigorously controlled simultaneous experiments of vector competence between *I. scapularis* and *I. dammini* have been reported, there appears to be no difference between them regarding vectorial ability for this spirochete in the laboratory (W. Burgdorfer, personal communication). *I. scapularis* from feral animals in nature (Magnarelli et al. 1986, Luckhart et al. 1991) and from vegetation (Levine et al. 1989) have also been shown to be infected with *B. burgdorferi*. We have detected *B. burgdorferi*-infected *I. scapularis* in nature from animals and vegetation in Georgia. A Georgia strain of *B. burgdorferi* has also been inoculated into hamsters and subsequently transmitted by *I. scapularis* to mice (J.H.O., F. W. Chandler, M. P. Luttrell, A.M.J., D. E. Stallknecht, B. S. McGuire, H.J.H., G. A. Cummins & R. S. Lane, unpublished data).

Significance of Results. There are many reasons for needing to know the relatedness of *I. scapularis* and *I. dammini*. The knowledge that they are conspecific should alert physicians and public health officials in geographic regions not usually considered at risk for Lyme disease that, if *I. scapularis* is present, at least one part of the Lyme disease equation is present. The ecology of different geographic populations of *I. scapularis* likely will vary, however, reflecting the local density-dependent and independent factors. Although much knowledge of Lyme disease and *I. dammini* gained in the northeastern and north-central United States can be extrapolated to the southern regions, detailed investigations will be needed to consider different ecological parameters. Clearly, developmental periods of ticks, availability of hosts for ticks, and reservoir hosts to the spirochete are likely to vary among different regions.

There are many basic evolutionary questions regarding the relatedness of tick species in the *I. ricinus/persulcatus* species complex. We are in the process of assessing genetic relatedness of species in this complex, which includes the four most important Lyme disease vectors: *I. scapularis* (including *I. dammini*), *I. pacificus* (North America), *I. ricinus* (L.) (Europe), and *I. persulcatus* Schulze (Asia). One approach used to study species-diagnostic differences is based on a ribosomal DNA (rDNA) internal transcribed spacer segment (ITS2), which has been used successfully in distinguishing sibling species of anopheline mosquitoes (Porter & Collins 1991). Therefore, we decided to use sequences of ITS2 clones of polymerase chain reaction (PCR)-amplified DNA from ticks from various parts of the world. Concurrently, as already noted, in collaboration with D. Wesson and F. H. Collins (Centers for Disease Control, Atlanta), these sequences are being used to determine species relationships of several geographic populations of *I. dammini* (including ticks from Massachusetts, New York, New Jersey, Maryland, and Wisconsin), *I. scapularis* (Georgia, North Carolina), and *I. pacificus* (Arizona, California). Analysis of sequence variation (e.g., neighbor-joining method, UPGMA) indicates that *I. pacificus* is a distinct species but that *I. scapularis* and *I. dammini* are conspecific (e.g., undifferentiated members of a cohesive gene pool) (Templeton 1989).

Conclusion. Although there are numerous definitions of a species (Mayr 1963, White 1978, Templeton 1989), all involve divergence in some characters. Because no major divergence could be demonstrated between *I. scapularis* and *I. dammini* in experiments involving hybridization, assortative mating, morphometrics, chromosomes, isozymes, and life cycles by us or divergences in host preferences, vector competencies, and DNA sequences by others, we conclude that *I. dammini* is not a valid species, and that most ticks identified as such are probably *I. scapularis*. Based on Article 23 of the *International Code of Zoological Nomenclature* (1985), the name *Ixodes scapularis* Say, 1821, has priority over the name *Ixodes dammini* Spielman, Clifford, Piesman & Corwin, 1979, and *I. dammini* is therefore relegated to a junior subjective synonym of *I. scapularis*.

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