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Comparative genetic diversity of Lyme disease bacteria in Northern Californian ticks and their vertebrate hosts

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ABSTRACT

Vector-borne pathogens are transmitted between vertebrate hosts and arthropod vectors, two immensely different environments for the pathogen. There is further differentiation among vertebrate hosts that often have complex, species-specific immunological responses to the pathogen. All this presents a heterogeneous environmental and immunological landscape with possible consequences on the population genetic structure of the pathogen. We evaluated the differential genetic diversity of the Lyme disease pathogen, *Borrelia burgdorferi*, in its vector, the western black-legged tick (*Ixodes pacificus*), and in its mammal host community using the 5S–23S rRNA intergenic spacer region. We found differences in haplotype distribution of *B. burgdorferi* in tick populations from two counties in California as well as between a sympatric tick and vertebrate host community. In addition, we found that three closely related haplotypes consistently occurred in high frequency in all sample types. Lastly, our study found lower species diversity of the *B. burgdorferi* species complex, known as *B. burgdorferi* sensu lato, in small mammal hosts versus the tick populations in a sympatric study area.

Introduction

Lyme disease, the most common vector-borne disease in North America, is caused by the bacterial spirochete, *Borrelia burgdorferi* (Spirochaetacae; Johnson et al., 1984). Though only described in 1984 (Johnson et al., 1984), molecular evidence suggests that North American populations of *B. burgdorferi* diverged from European populations several million years ago and have been well established in the northeastern, midwestern and far-western United States for some time (Margos et al., 2008). The disease vector and the bacterial pathogen feed on and cause infection in many vertebrate species, respectively (LoGiudice et al., 2003; Hanincova et al., 2006; Castro and Wright, 2007). The vector, *Ixodes* spp. ticks, feed on a suite of vertebrate hosts for blood meals including various species of mammals, birds and lizards (Furman and Loomis, 1984; Kurtenbach et al., 2006) and the bacteria is maintained and transmitted by many of these hosts back to the vector (Donahue et al., 1987; Mather et al., 1989; Telford et al., 1990; Lane and Brown, 1991; Brown and Lane, 1996; Lane et al., 2005; Brunner et al., 2008; Salkeld et al., 2008).

The life cycle of the pathogen requires both the tick vector and vertebrate host because there is no vertical transmission in either. The bacteria reside in the tick midgut until the tick attaches to a suitable host. The pathogen then replicates and migrates to the tick's salivary gland before entering a vertebrate host where it disseminates via the blood and causes multi-systemic infection in the host (Piesman et al., 1990, 1991; Barthold et al., 1991). The tick vector feeds three times, once during each of its life stages: larvae, nymph and adult. After each blood meal, the tick molts into the next stage or, in the case of adults, reproduces. In North America, nymphal stages of *Ixodes scapularis* Ixodidae in the East and *I. pacificus* in the West are the principal vectors of the pathogen to humans (Barbour and Fish, 1993; Clover and Lane, 1995).

Because *B. burgdorferi* infects many vertebrate species, it must contend with immunological factors in each host species it infects. Some host species, such as western fence lizards (*Sceloporus occidentalis*) and alligator lizards (*Elgaria multicarinata*) do not maintain the bacteria and kill the bacteria in the feeding tick (Lane and Quistad, 1998; Wright et al., 1998), whereas other pathogen reservoir hosts transmit the bacteria to feeding ticks with varying levels of efficiency (Donahue et al., 1987; Brown and Lane, 1996; Eisen et al., 2003; LoGiudice et al., 2003; Brunner et al., 2008). For

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instance, mule deer and birds have not been shown to be a viable pathogen reservoir in coastal woodlands of California (Ullmann et al., 2003; Eisen et al., 2004). Furthermore, certain pathogen genotypes may be better adapted to invade particular host species (Brisson, 2004; Brisson and Dykhuizen, 2006). Immunologically speaking, *B. burgdorferi* faces a heterogeneous host "landscape" that may lead to differential transmission and survival of particular bacterial genotypes or lineages (Kurtenbach et al., 2002a).

DNA sequence data from a neutral genetic marker can be used to compare the diversity of *B. burgdorferi* and has been used to determine population structure and infer gene flow within and between regions (Postic et al., 1994; Humphrey et al., 2010; Coipan et al., 2013). The 5S (*rrf*)–23S (*rrl*) intergenic spacer region is a tandemly repeated sequence that is unique to *B. burgdorferi* sensu lato and has been used to differentiate and identify different isolates of *Borrelia* spirochetes in many different sample types including ticks (Postic et al., 1994; Humphrey et al., 2010), blood meal hosts (Chu et al., 2008) and patients (Postic et al., 1998; Rijpkema et al., 2009). This is in contrast with other markers such as the *ospA* or *ospC* genes are not ideal for phylogenetic analyses of inferences because of frequent recombination events (Haven et al., 2011). Previous studies from the northeastern or midwestern US have examined the genetic structure of *B. burgdorferi* with other genes and found limited genetic structure at a local scale but evidence of regional barriers to gene flow (Qiu et al., 2002; Humphrey et al., 2010). This is the first study to comparatively examine haplotype diversity in *Ixodes pacificus* and their vertebrate hosts in the same spatiotemporal context. This study is unique in this regard and can thereby provide valuable insights into key elements of the population genetic history and ecology of pathogen transmission. The genotype diversity of *B. burgdorferi* present in ticks and its vertebrate hosts should theoretically match because they are linked in the reproduction and transmission of the pathogen. If they do not, then it suggests that there are unidentified pathogen reservoirs or differential transmission rates of particular isolates (Girard et al., 2009).

Within its North American distribution, Lyme disease is caused by *B. burgdorferi* sensu stricto (*B. burgdorferi* s.s.), the only confirmed cause of Lyme disease in North America (Margos et al., 2010; Wormser et al., 2008). In Europe, two other genospecies *B. afzelii* and *B. garinii* also cause disease in humans. These three genospecies, along with 14 other described genospecies and several additional, undescribed isolates are collectively referred to as the *B. burgdorferi* sensu lato (*B. burgdorferi* s.l.) complex (reviewed in Stanek and Reiter, 2011). In North America, members of the *B. burgdorferi* s.l. species complex that are not *B. burgdorferi* s.s. have, to date, not been shown to cause disease in humans in North America, but see Girard et al. (2011). It has been proposed that areas with greater genetic diversity of *B. burgdorferi* s.l. result from a higher number of closed enzootic cycles between ticks and particular host species that generate more habitat "niches" for the bacterial pathogen (Margos et al., 2010). This study examines the genetic diversity of a non-coding IGS locus in *B. burgdorferi* s.l. from a large sample of *I. pacificus* and its vertebrate host community in northern California and evaluates the ecological significance of these patterns.

Given the challenge of capturing all possible hosts in a community (and thus all pathogen reservoirs), we expected the genetic diversity of *B. burgdorferi* that we detect in tick hosts, to be a subset of the diversity that we find in the tick population.

Methods

Field collections

Collections of *I. pacificus* ticks took place in two adjacent northern California counties: Marin and Sonoma. Sampling in Marin County took place from 2006 to 2009 at two oak woodland sites, China Camp State Park (38°0′9.50″N, 122°28′2.53″W) and Marin Municipal Water District (37°58′5.39″N, 122°36′15.20″W). Field sampling in Sonoma County was conducted in 2006 and 2007 in several state parks and reserves, including Jack London State Historic Park (38°21′24.12″N, 122°32′38.4″W), Annadel State Park (38°27′7.2″N, 122°38′2.4″W), Sugarloaf Ridge State Park (38°26′16.30″N, 122°30′51.55″W), Sonoma State University Fairfield Osborn Preserve (38°20′56.12″N, 122°35′44.56″W) and Audubon Canyon Ranch Bouverie Preserve (38°21′51.69″N, 122°30′35.9″W) (Swei et al., 2011a). Sampled sites were in oak woodland habitat and chosen from a mixture of randomly selected plots (Meentemeyer et al., 2008). All ticks were collected by drag sampling using a 1 m^2 white flannel cloth attached to a wooden dowel (Swei et al., 2011a). Drag cloths were checked every 15 m and all attached ticks were removed and stored in 70% ethanol for lab identification and DNA extraction.

Tick host sampling targeted small mammals because local lizards are non-competent pathogen reservoirs of *B. burgdorferi* s.l. and birds have not been shown to be important reservoirs in north-coastal California (Eisen et al., 2004). Small mammal hosts of *I. pacificus* were collected from Marin County in 2006, 2007 and 2008 by live-trapping with extra-long Sherman traps (7.6 × 9.5 × 30.5 cm; H.B. Sherman Traps, Tallahassee, FL) on the tick collection plots (Swei et al., 2011b). Detailed animal handling methods are provided in Swei et al. (2011b). Briefly, all trapped animals were anesthetized with a 5% solution of isoflurane before two 2-mm ear punch biopsies were collected, one from each ear. Tissue samples were stored in 70% ethanol and refrigerated at −20 °C until extracted. Animals were allowed to revive before being released at the point of capture. Tick–host sampling was not conducted in Sonoma County.

Laboratory analyses

DNA from all *I. pacificus* nymphs and vertebrate tissue samples was extracted using a Qiagen DNeasy Kit (Qiagen, Foster City, CA, USA) following the manufacturer's instructions. All tick samples were then screened for infection with *B. burgdorferi* s.l. using real-time quantitative PCR techniques detailed in Swei et al. (2011b). Positive samples were PCR-amplified and sequenced at the 5S–23S *rrf–rrl* intergenic spacer (IGS) rDNA region (Lane et al., 2004). Much of the existing genetic work in western North America, including species descriptions, have used this neutral IGS marker for its utility to differentiate genospecies and reveal genetic differentiation at small geographic scales (Coipan et al., 2013; Postic et al., 1994; Rijpkema et al., 1995).

Cycle-sequencing reactions were performed in both forward and reverse directions using the ABI BigDye Terminator Kit v3.1 (Applied Biosystems, Foster City, CA). Cycle-sequencing reaction products were purified using Sephadex columns and then analyzed on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA), following the protocol provided in Lane *et al.* (2004). Forward and reverse sequences were assembled and edited in Sequencher 4.7 (Gene Codes Corp., Ann Arbor, MI).

Alignment and phylogenetic analyses

Sequences were aligned using MAFFT (Katoh et al., 2009). Maximum likelihood (ML) analyses were performed using RAxML 2.2.3 (Stamatakis et al., 2008) via the CIPRES portal (Miller et al., 2009) to generate a topology with boostrap support values (100 pseudoreplicates). We considered a clade to be supported if bootstrap values were greater than 50%, and to be strongly supported if greater than 70% (Hillis and Bull, 1993). Phylogenetic trees were visualized using FigTree v1.0 (Rambaut, 2006). Examination of the