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Lyme disease risk not amplified in a species-poor vertebrate community: Similar *Borrelia burgdorferi* tick infection prevalence and OspC genotype frequencies

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ABSTRACT

The effect of biodiversity declines on human health is currently debated, but empirical assessments are lacking. Lyme disease provides a model system to assess relationships between biodiversity and human disease because the etiologic agent, *Borrelia burgdorferi*, is transmitted in the United States by the generalist black-legged tick (*Ixodes scapularis*) among a wide range of mammalian and avian hosts. The 'dilution effect' hypothesis predicts that species-poor host communities dominated by white-footed mice (*Peromyscus leucopus)* will pose the greatest human risk because *P. leucopus* infects the largest numbers of ticks, resulting in higher human exposure to infected *I. scapularis* ticks. *P. leucopus*-dominated communities are also expected to maintain a higher frequency of those *B. burgdorferi* outer surface protein C (ospC) genotypes that this host species more efficiently transmits ('multiple niche polymorphism' hypothesis). Because some of these genotypes are human invasive, an additive increase in human disease risk is expected in species-poor settings. We assessed these theoretical predictions by comparing *I. scapularis* nymphal infection prevalence, density of infected nymphs and *B. burgdorferi* genotype diversity at sites on Block Island, RI, where *P. leucopus* dominates the mammalian host community, to species-diverse sites in northeastern Connecticut. We found no support for the dilution effect hypothesis; *B. burgdorferi* nymphal infection prevalence was similar between island and mainland and the density of *B. burgdorferi* infected nymphs was higher on the mainland, contrary to what is predicted by the dilution effect hypothesis. Evidence for the multiple niche polymorphism hypothesis was mixed: there was lower ospC genotype diversity at island than mainland sites, but no overrepresentation of genotypes with higher fitness in *P. leucopus* or that are more invasive in humans. We conclude that other mechanisms explain similar nymphal infection prevalence in both communities and that high ospC genotype diversity can be maintained in both species-poor and species-rich communities.

1. Introduction

The role of biodiversity in buffering against human infectious disease has been proposed as an ecosystem service widely applicable to a number of infectious diseases (Daszak et al., 2001; Diaz et al., 2006; Keesing et al., 2010; Ostfeld and LoGiudice, 2003). Originally developed within the field of malaria epidemiology as the concept of 'zooprophylaxis' (Dobson et al., 2006; Macdonald, 1957), the proposed mechanism is that more diverse host communities may include one or more hosts that are inefficient reservoirs and act as pathogen sinks, resulting in a net loss of the pathogen in the system ('dilution effect' hypothesis, Norman et al., 1999; Schmidt and Ostfeld, 2001). Lyme disease provides an excellent model system to assess the relationship between biodiversity and human health. The etiologic agent of Lyme disease, *Borrelia burgdorferi* sensu lato is transmitted by generalist vectors of the *Ixodes ricinus* species complex (*I. ricinus* in Europe, *I. persulcatus* in Asia, *I. pacificus* on the western coast of North America and *I.*

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scapularis in eastern North America) among a wide range of evolutionarily divergent vertebrate hosts, including mammalian and avian species, which vary in their propensity to acquire and transmit the pathogen, or reservoir competence (Kurtenbach et al., 2006). Here we focus on the epidemiological and ecological context of Lyme disease in eastern North America. *I. scapularis* has three post-egg stages that are obligate parasites: the larval and nymphal stages feed on a range of hosts of varying reservoir competence, while the adult stage feeds almost exclusively on white-tailed deer (*Odocoileus virginianus*), which is a reservoir incompetent host (Battaly and Fish, 1993; Brinkerhoff et al., 2011; Brisson and Dykhuizen, 2004; Fish and Daniels, 1990; Giardina et al., 2000; Ginsberg et al., 2005; Hanincova et al., 2006; Keesing et al., 2009; LoGiudice et al., 2003; Mather et al., 1989; Rand et al., 1993). The white-footed mouse (*Peromyscus leucopus*) is considered the primary reservoir host in the United States because of its abundance, high tick burden and high reservoir competence (Donahue et al., 1987; Lane et al., 1991; Mather et al., 1989). Thus, in the Lyme disease model system, the dilution effect hypothesis predicts higher *B. burgdorferi* abundance when host communities are dominated by *P. leucopus* (LoGiudice et al., 2003).

Heterogeneous host communities may also increase pathogen diversity by providing an increased variety of ecological ‘niches’ that allow the maintenance of polymorphisms within populations (‘multiple niche polymorphism’ hypothesis, hereafter MNP hypothesis) (Levene, 1953). MNP has been proposed as a form of balancing selection that can maintain the polymorphism in the gene coding for the outer surface protein C (ospC) of *B. burgdorferi* (Brisson and Dykhuizen, 2004, 2006; Kurtenbach et al., 2006). OspC is a highly variable and important antigenic factor in vertebrate hosts (Fingerle et al., 1995; Schwan and Piesman, 2000; Schwan et al., 1995). OspC genetic diversity is classified into major groups of alleles (A–X), defined as a group of alleles that are less than 2% different in protein sequence and more than 8% different from alleles in other major groups. Of the 24 ospC major groups (hereafter, OMGs) found worldwide, 17 occur at fairly even frequencies in every well sampled population in the northeastern United States (Brisson et al., 2012; Brisson and Dykhuizen, 2004; Qiu et al., 2002; Rudenko et al., 2013; Seinost et al., 1999; Wang et al., 1999). Variation in the ability of OMGs to infect different vertebrate species has been proposed as a result of diversifying selection acting on the ospC that may drive *B. burgdorferi’s* host specialization (Brisson and Dykhuizen, 2004, 2006). Thus, the MNP hypothesis predicts that *B. burgdorferi* diversity would be lower in species-poor host communities and that genotype frequencies will vary in accordance to the species composition of the community (Brisson and Dykhuizen, 2006). Because some ospC genotypes are more persistent and more efficiently transmitted by *P. leucopus* and are more likely to cause invasive disease in humans, an additional prediction is that *P. leucopus*-dominated communities represent increased human disease risk (Derdakova et al., 2004; Dykhuizen et al., 2008; Hanincova et al., 2013; Hanincova et al., 2008; Seinost et al., 1999; Wang et al., 2002; Wormser et al., 1999).

Debate exists as to whether reduced biodiversity alone can explain reduced human infection risk (Lafferty and Wood, 2013; Ogden and Tsao, 2009; Ostfeld, 2013; Ostfeld and Keesing, 2013; Randolph and Dobson, 2012; Wood and Lafferty, 2013). Studies supporting the dilution effect hypothesis have mostly relied on mathematical modeling based on data from one location (LoGiudice et al., 2003) or on comparing differently sized forest patches used as proxies for host community composition (Allan et al., 2003). The only empirical test involving experimental removal of non-competent lizard hosts in California did not support predictions of the dilution effect (Swei et al., 2011) and a study sampling host communities across a fragmentation gradient found a weak association between Lyme disease risk and small mammal host diversity and did not find a relationship between Lyme disease risk and fragment size (LoGiudice et al., 2008). A recent study examines the dilution effect along a range of divergent island host communities and found that the interaction between the relative abundance of small mammal hosts and species richness may result in both dilution and amplification effects (Werden et al., 2014).

The mechanism by which ospC diversity is maintained is also debated and mixed theoretical and empirical evidence exists as to whether each host species transmits a consistent subset of genotypes, a prerequisite for the maintenance of ospC diversity by MNP (Anderson and Norris, 2006; Brisson and Dykhuizen, 2004; Hanincova et al., 2006; Vuong et al., 2013). Furthermore, even if certain hosts transmit a subset of genotypes at a higher frequency than others, whether any selection pressure exerted by host specialization can overcome other selective or non-selective forces and significantly influence genotype diversity in a community remains to be tested (Brisson et al., 2012; Haven et al., 2011; Kurtenbach et al., 2006).

The dilution effect and MNP hypotheses jointly predict additive increases in human Lyme disease risk in species-poor, *P. leucopus*-dominated, communities (Brisson and Dykhuizen, 2006). These predictions have not been empirically tested. Alternatively, highly diverse vertebrate host communities that foster high diversity of pathogens could lead to an increased risk of vector-borne and zoonotic disease over space and time (Jones et al., 2008). In this study we empirically evaluated the impacts of host diversity on *B. burgdorferi* nymphal infection as well as on *B. burgdorferi* OMG genotypic diversity. We focused on the nymphal stage because it is the only tick life stage that has a significant role as a vector for *B. burgdorferi* human infection in eastern North America (Falco et al., 1999; Mather et al., 1996; Pepin et al., 2012). More specifically, our goal was to assess whether predictions from the dilution effect and the MNP hypotheses were upheld in a comparative study of an island community with low host diversity dominated by *P. leucopus* (Block Island, Rhode Island) and a more host diverse mainland community in northeastern Connecticut. We sought to test the following three specific predictions of the dilution and MNP hypotheses: (1) *B. burgdorferi* infection prevalence in *I. scapularis* nymphs and the density of infected nymphs are higher in the low diversity community than the high diversity community, (2) *B. burgdorferi* OMG diversity is lower and OMG frequency distribution is different in island vs. mainland communities, and (3) OMGs shown to be more efficiently transmitted by *P. leucopus* are more prevalent in the lower diversity community.

2. Methods

2.1. Study design

The study was conducted in peridomestic and natural sites on Block Island, Rhode Island (hereafter ‘island’), and in the towns of Hampton, Mansfield, and Willington in northeastern Connecticut (hereafter ‘mainland’). Block Island is a 25.2 km^2 landmass 23 km south of mainland Rhode Island. Peridomestic sites were residential properties adjacent to deciduous forest and shrublands in Connecticut; only shrublands surrounded properties on Block Island. Deciduous forest, the most suitable habitat type for *I. scapularis* in the mainland, is limited on the island to a 4 ha site (Enser, 2000); thus most tick habitat on Block Island is restricted to shrublands and shrub edges with sufficient leaf litter accumulation for tick survival (Finch et al., 2014). The mammalian community on the island is composed exclusively of the white-footed mouse (*P. leucopus*), house mouse (*Mus musculus*), meadow vole (*Microtus pennslyvanica*), brown rat (*Rattus norvegicus*), muskrat (*Ondatra zibethicus*), and white-tailed deer (*O. virginianus*) (Comings, 2006;